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(54) Title: NOVEL HISTAMINE H2 RECEPTOR (57) Abstract The present invention provides a novel histamine H2 receptor (H2RH) and polynucleotides which identify and encode H2RH. The invention also provides genetically engineered expression vectors and host cells comprising the nucleic acid sequences encoding H2RH and a method for producing H2RH. The invention also provides for agonists, antibodies, or antagonists specifically binding H2RH, and their use, in the prevention and treatment of diseases in which H2RH is expressed. Additionally, the invention provides for the use of antisense molecules to polynucleotides encoding H2RH for the treatment of diseases associated with the expression of H2RH. The invention also provides diagnostic assays which utilize the polynucleotide, or fragments or the complement thereof, and antibodies specifically binding H2RH.		

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NOVEL HISTAMINE H2 RECEPTOR

TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of a novel histamine H2 receptor and to the use of these sequences in the diagnosis, prevention, and treatment of various infectious and inflammatory conditions.

BACKGROUND ART

Histamine is found in mast cells in all peripheral tissues. It is released via degranulation of mast cells as part of allergic and inflammatory responses to allergens, infection or trauma. Chronic inflammation can contribute to degradation of host tissue and will eventually lead to tissue destruction and to clinical disease associated with loss of organ function. Histamine is widely distributed in both neuronal and non-neuronal cells and has been implicated in a variety of CNS functions including arousal and analgesia.

In low concentrations, histamine mediates inflammation and allergies through the activation of the H1 receptor. The H1 receptor is widely distributed, regulates the contraction of smooth muscles of the intestine, trachea, bladder and blood vessels. The H1 receptor is also associated with the adrenal medulla, vascular endothelium, heart, cerebral cortex, cerebellum and spinal cord. In high concentrations, histamine regulates the release of gastric acid from parietal cells of the digestive system through activation of the histamine H2 receptor. The H2 receptor is found at high levels in heart and stomach and has limited distribution in smooth muscle, the uterus, and cells of the immune system. Both the H1 and the H2 receptors are seven transmembrane, G-protein coupled receptors (T7G).

T7G receptors are characterized by their seven hydrophobic domains which span the plasma membrane and form a bundle of antiparallel alpha helices. The transmembrane segments (TMS) are designated by roman numerals I to VII and account for structural and functional features of the receptor. In most cases, the bundle of helices forms a binding pocket; however, when the binding site must accommodate more bulky molecules, the extracellular N-terminal segment or one or more of the three extracellular loops participate in binding and in subsequent induction of conformational change in intracellular portions of the receptor. The activated receptor, in turn, interacts with an intracellular G-protein complex which mediates further intracellular signaling activities, generally interaction with guanine

nucleotide binding (G) proteins and the production of second messengers such as cyclic AMP (cAMP), phospholipase C, inositol triphosphate or ion channel proteins.

The amino-terminus of the T7G is extracellular, of variable length and often glycosylated, while the carboxy-terminus is cytoplasmic and generally phosphorylated.

5 Extracellular loops of the T7G alternate with intracellular loops and link the TMS. The most conserved domains of T7Gs are the transmembrane regions and the first two cytoplasmic loops. Taxonomic groups and their characteristics are reviewed in Bolander, F.F. (1994; Molecular Endocrinology, Academic Press, San Diego CA), and some structural and functional information is summarized in Watson, S. and S. Arkinstall (1994; The G-Protein
10 Linked Receptor Facts Book, Academic Press, San Diego CA).

Much of the known information on histamine receptors, their conformations and activities is based on observations made following the administration of antagonists. For example, conventional antihistamines block the actions of histamine on smooth muscle of blood vessels, gut, or bronchi, but they do not inhibit histamine-stimulated gastric acid
15 secretion. This observation led to further study of histamine receptors and the development of specific classes of antagonists for the different types of receptors. The conventional histamine receptor which is blocked by classic antihistamines is the known H-1 receptor whereas the gastric parietal cell or H-2 receptor (H2R) is not affected. H1 antagonists are used clinically in the treatment of allergic, asthmatic and anaphylactic reactions. The H2R
20 antagonists are potent inhibitors of gastric acid secretion, exhibit some structural similarities to histamine and to each other, and are generally effective in healing and reducing the occurrence of gastric ulcers, particularly duodenal ulcers.

The H2R antagonist cimetidine, which shares the imidazole ring with histamine and bears a side chain containing a cyanoguanidine group, inhibits acid secretion by 70-80% and
25 also reduces secretion in response to histamine, caffeine, insulin, hypoglycemia, and gastrin. Very few side effects are associated with cimetidine administration although slight and reversible increases in serum aminotransferase, creatinine, and serum prolactin levels are reported. Other inhibitors such as ranitidine, famotidine and nizatidine are about 6-10 times as potent as cimetidine in inhibiting gastric acid secretion and are also effective in the healing
30 and prevention of duodenal ulcers. Structurally, ranitidine is a substituted aminomethylfuran,

whereas famotidine and nizatidine have thiazole rings. As with cimetidine, a few mild side effects have been noted (Isselbacher, K.J. et al. (1994) Harrison's Principles of Internal Medicine, McGraw Hill, New York, NY).

Evidence exists that several diseases may be caused by G protein and T7G mutations which cause either loss- or gain-of-function (Spiegel, A.M. (1995) *Nature Med.* 1:522-24). For example, a gain-of-function mutation of H2R which mimics the effects of excess histamine could actually cause disease. A recent report linked the knockout of the mouse G protein $\alpha i2$ gene to a lethal form of ulcerative colitis similar to the human disease. In this study, the mutation eventually led to development of adenocarcinoma in the mouse colon (Rudolph U. et. al. (1995) *Nature Genet.* 10:141-8).

The discovery of polynucleotides encoding novel histamine receptors, and the molecules themselves, presents the opportunity to investigate inflammatory conditions. Discovery of molecules related to histamine H2 receptors satisfies a need in the art by providing molecules which can be used to screen for and develop more effective antihistamines or other therapeutic compositions useful in the treatment of various infectious and inflammatory conditions.

DISCLOSURE OF THE INVENTION

The present invention features a novel histamine H2 receptor, hereinafter designated H2RH and characterized as having chemical and structural homology to histamine receptors. Accordingly, the invention features a substantially purified H2RH having the amino acid sequence, SEQ ID NO:1.

One aspect of the invention features isolated and substantially purified polynucleotides that encode H2RH. In a particular aspect, the polynucleotide is the nucleotide sequence of SEQ ID NO:2.

The invention also relates to a polynucleotide sequence comprising the complement of SEQ ID NO:2 or variants thereof. In addition, the invention features polynucleotide sequences which hybridize under stringent conditions to SEQ ID NO:2.

The invention additionally features nucleic acid sequences encoding polypeptides, oligonucleotides, peptide nucleic acids (PNA), fragments, portions or antisense molecules thereof, and expression vectors and host cells comprising polynucleotides that encode H2RH.

The present invention also features antibodies which bind specifically to H2RH, and pharmaceutical compositions which modulate the activity of H2RH. The invention specifically features the identification and use of antagonists of H2RH.

BRIEF DESCRIPTION OF DRAWINGS

5 Figures 1A, 1B, 1C and 1D shows the amino acid sequence (SEQ ID NO:1) and nucleic acid sequence (SEQ ID NO:2) of the novel histamine H2 inhibitor, H2RH. The alignment was produced using MacDNASIS PRO™ software (Hitachi Software Engineering Co., Ltd., San Bruno, CA).

10 Figures 2A, 2B, 2C, 2D and 2E shows the amino acid sequence alignments among H2RH (SEQ ID NO:1), canine H2R (GI 163952; SEQ ID NO:3), human H2R (GI 184088; SEQ ID NO:4), Cavia H2R (GI 791239; SEQ ID NO:5) rat H2R (GI 236184; SEQ ID NO:6), human alpha 1C adrenergic receptor (GI 927211; SEQ ID NO:7), and human 5-HT2B serotonin receptor (GI 475198; SEQ ID NO:8). The alignment was produced using the multisequence alignment program of DNASTAR™ software (DNASTAR Inc, Madison WI).

15 Figure 3 shows the hydrophobicity plot (MacDNASIS PRO software) for H2RH, SEQ ID NO: 1; the positive X axis reflects amino acid position, and the negative Y axis, hydrophobicity.

 Figure 4 shows the hydrophobicity plot for canine H2R, SEQ ID NO:3.

MODES FOR CARRYING OUT THE INVENTION

20 Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the
25 appended claims.

 It must be noted that as used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to “a host cell” includes a plurality of such host cells, reference to the “antibody” is a reference to one or more antibodies and equivalents thereof known to
30 those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are reported in the publications which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

"Nucleic acid sequence" as used herein refers to an oligonucleotide, nucleotide, or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single- or double-stranded, and represent the sense or antisense strand. Similarly, "amino acid sequence" as used herein refers to an oligopeptide, peptide, polypeptide, or protein sequence, and fragments or portions thereof, and to naturally occurring or synthetic molecules.

Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms, such as "polypeptide" or "protein" are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule.

"Peptide nucleic acid", as used herein, refers to a molecule which comprises an oligomer to which an amino acid residue, such as lysine, and an amino group have been added. These small molecules, also designated anti-gene agents, stop transcript elongation by binding to their complementary strand of nucleic acid (Nielsen, P.E. et al. (1993) Anticancer Drug Des. 8:53-63).

H2RH, as used herein, refers to the amino acid sequences of substantially purified H2RH obtained from any species, particularly mammalian, including bovine, ovine, porcine, murine, equine, and preferably human, from any source whether natural, synthetic, semi-synthetic, or recombinant.

"Consensus", as used herein, refers to a nucleic acid sequence which has been

resequenced to resolve uncalled bases, or which has been extended using XL-PCR™ (Perkin Elmer, Norwalk, CT) in the 5' and/or the 3' direction and resequenced, or which has been assembled from the overlapping sequences of more than one Incyte clone using the GELVIEW™ Fragment Assembly system (GCG, Madison, WI), or which has been both
5 extended and assembled.

A "variant" of H2RH, as used herein, refers to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. More rarely, a variant may have "nonconservative" changes, e.g.,
10 replacement of a glycine with a tryptophan. Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software.

15 A "deletion", as used herein, refers to a change in either amino acid or nucleotide sequence in which one or more amino acid or nucleotide residues, respectively, are absent.

An "insertion" or "addition", as used herein, refers to a change in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid or nucleotide residues, respectively, as compared to the naturally occurring molecule.

20 A "substitution", as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

The term "biologically active", as used herein, refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic
25 H2RH, or any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The term "agonist", as used herein, refers to a molecule which, when bound to H2RH, causes a change in H2RH which modulates the activity of H2RH. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to H2RH.

30 The terms "antagonist" or "inhibitor", as used herein, refer to a molecule which, when

bound to H2RH, blocks or modulates the biological or immunological activity of H2RH. Antagonists and inhibitors may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to H2RH.

5 The term “modulate”, as used herein, refers to a change or an alteration in the biological activity of H2RH. Modulation may be an increase or a decrease in protein activity, a change in binding characteristics, or any other change in the biological, functional or immunological properties of H2RH.

10 The term “mimetic”, as used herein, refers to a molecule, the structure of which is developed from knowledge of the structure of H2RH or portions thereof and, as such, is able to effect some or all of the actions of PE-60-like molecules.

15 The term “derivative”, as used herein, refers to the chemical modification of a nucleic acid encoding H2RH or the encoded H2RH. Illustrative of such modifications would be replacement of hydrogen by an alkyl, acyl, or amino group. A nucleic acid derivative would encode a polypeptide which retains essential biological characteristics of the natural molecule.

The term “substantially purified”, as used herein, refers to nucleic or amino acid sequences that are removed from their natural environment, isolated or separated, and are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated.

20 “Amplification” as used herein refers to the production of additional copies of a nucleic acid sequence and is generally carried out using polymerase chain reaction (PCR) technologies well known in the art (Dieffenbach, C.W. and GS Dveksler (1995) PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview, NY).

25 The term “hybridization”, as used herein, refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

30 The term “hybridization complex”, as used herein, refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen binds between complementary G and C bases and between complementary A and T bases; these hydrogen bonds may be further stabilized by base stacking interactions. The two complementary nucleic acid sequences hydrogen bond in an antiparallel configuration. A hybridization

complex may be formed in solution (e.g., C_0t or R_0t analysis) or between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., membranes, filters, chips, pins or glass slides to which cells have been fixed for in situ hybridization).

5 The terms “complementary” or “complementarity”, as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. For example, for the sequence “A-G-T” binds to the complementary sequence “T-C-A”. Complementarity between two single-stranded molecules may be “partial”, in which only some of the nucleic acids bind, or it may be complete when total complementarity exists
10 between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands.

 The term “homology”, as used herein, refers to a degree of complementarity. There
15 may be partial homology or complete homology (i.e., identity). A partially complementary sequence is one that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid; it is referred to using the functional term “substantially homologous.” The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution
20 hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe will compete for and inhibit the binding (i.e., the hybridization) of a completely homologous sequence or probe to the target sequence under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to
25 one another be a specific (i.e., selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% identity); in the absence of non-specific binding, the probe will not hybridize to the second non-complementary target sequence.

 As known in the art, numerous equivalent conditions may be employed to comprise
30 either low or high stringency conditions. Factors such as the length and nature (DNA, RNA,

base composition) of the sequence, nature of the target (DNA, RNA, base composition, presence in solution or immobilization, etc.), and the concentration of the salts and other components (e.g., the presence or absence of formamide, dextran sulfate and/or polyethylene glycol) are considered and the hybridization solution may be varied to generate conditions of either low or high stringency different from, but equivalent to, the above listed conditions.

The term “stringent conditions”, as used herein, is the “stringency” which occurs within a range from about $T_m - 5^\circ\text{C}$ (5°C below the melting temperature (T_m) of the probe) to about 20°C to 25°C below T_m . As will be understood by those of skill in the art, the stringency of hybridization may be altered in order to identify or detect identical or related polynucleotide sequences.

The term “antisense”, as used herein, refers to nucleotide sequences which are complementary to a specific DNA or RNA sequence. The term “antisense strand” is used in reference to a nucleic acid strand that is complementary to the “sense” strand. Antisense molecules may be produced by any method, including synthesis by ligating the gene(s) of interest in a reverse orientation to a viral promoter which permits the synthesis of a complementary strand. Once introduced into a cell, this transcribed strand combines with natural sequences produced by the cell to form duplexes. These duplexes then block either the further transcription or translation. In this manner, mutant phenotypes may be generated. The designation “negative” is sometimes used in reference to the antisense strand, and “positive” is sometimes used in reference to the sense strand.

The term “portion”, as used herein, with regard to a protein (as in “a portion of a given protein”) refers to fragments of that protein. The fragments may range in size from four amino acid residues to the entire amino acid sequence minus one amino acid. Thus, a protein “comprising at least a portion of the amino acid sequence of SEQ ID NO:1” encompasses the full-length human H2RH and fragments thereof.

“Transformation”, as defined herein, describes a process by which exogenous DNA enters and changes a recipient cell. It may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the host cell being transformed and may include, but is not

limited to, viral infection, electroporation, lipofection, and particle bombardment. Such "transformed" cells include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. They also include cells which transiently express the inserted DNA or RNA for limited periods of time.

The term "antigenic determinant", as used herein, refers to that portion of a molecule that makes contact with a particular antibody (i.e., an epitope). When a protein or fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to a given region or three-dimensional structure on the protein; these regions or structures are referred to as antigenic determinants. An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The terms "specific binding" or "specifically binding", as used herein, in reference to the interaction of an antibody and a protein or peptide, mean that the interaction is dependent upon the presence of a particular structure (i.e., the antigenic determinant or epitope) on the protein; in other words, the antibody is recognizing and binding to a specific protein structure rather than to proteins in general. For example, if an antibody is specific for epitope "A", the presence of a protein containing epitope A (or free, unlabeled A) in a reaction containing labeled "A" and the antibody will reduce the amount of labeled A bound to the antibody.

The term "sample", as used herein, is used in its broadest sense. A biological sample suspected of containing nucleic acid encoding H2RH or fragments thereof may comprise a cell, chromosomes isolated from a cell (e.g., a spread of metaphase chromosomes), genomic DNA (in solution or bound to a solid support such as for Southern analysis), RNA (in solution or bound to a solid support such as for northern analysis), cDNA (in solution or bound to a solid support), an extract from cells or a tissue, and the like.

The term "correlates with expression of a polynucleotide", as used herein, indicates that the detection of the presence of ribonucleic acid that is similar to SEQ ID NO:2 by northern analysis is indicative of the presence of mRNA encoding H2RH in a sample and thereby correlates with expression of the transcript from the polynucleotide encoding the protein.

“Alterations” in the polynucleotide of SEQ ID NO: 2, as used herein, comprise any alteration in the sequence of polynucleotides encoding H2RH including deletions, insertions, and point mutations that may be detected using hybridization assays. Included within this definition is the detection of alterations to the genomic DNA sequence which encodes H2RH (e.g., by alterations in the pattern of restriction fragment length polymorphisms capable of hybridizing to SEQ ID NO:2), the inability of a selected fragment of SEQ ID NO: 2 to hybridize to a sample of genomic DNA (e.g., using allele-specific oligonucleotide probes), and improper or unexpected hybridization, such as hybridization to a locus other than the normal chromosomal locus for the polynucleotide sequence encoding H2RH (e.g., using fluorescence in situ hybridization [FISH] to metaphase chromosomes spreads).

As used herein, the term “antibody” refers to intact molecules as well as fragments thereof, such as Fa, F(ab')₂, and Fv, which are capable of binding the epitopic determinant. Antibodies that bind H2RH polypeptides can be prepared using intact polypeptides or fragments containing small peptides of interest as the immunizing antigen. The polypeptide or peptide used to immunize an animal can be derived from the transition of RNA or synthesized chemically, and can be conjugated to a carrier protein, if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin and thyroglobulin. The coupled peptide is then used to immunize the animal (e.g., a mouse, a rat, or a rabbit).

The term “humanized antibody”, as used herein, refers to antibody molecules in which amino acids have been replaced in the non-antigen binding regions in order to more closely resemble a human antibody, while still retaining the original binding ability.

THE INVENTION

The invention is based on the discovery of a novel histamine H2 receptor, (H2RH), the polynucleotides encoding H2RH, and the use of these compositions for the diagnosis, prevention, or treatment of infectious and inflammatory conditions or diseases, including but not limited to those of the cardiovascular, digestive, immune, respiratory, reproductive, urinary or central nervous systems.

The nucleic acid sequence encoding the human H2RH of the present invention is found in Incyte Clone 1722180 from the bladder cDNA library (BLADNOT06). A consensus

sequence, SEQ ID NO:2, was derived from the extension and assembly of Incyte Clones 1278070 (SCORNOT03), 1722180 and 1722385 (BLADNOT06), and 1844864 (COLNNOT08). The sequence was first identified through a computer search of expressed sequences for amino acid sequence alignments.

5 In one embodiment, the invention encompasses the novel histamine H2 receptor, a polypeptide comprising the amino acid sequence of SEQ ID NO:1. H2RH is 454 amino acids in length and has a calculated molecular weight of 50587. Of five potential N-linked glycosylation sites, N₄ and N₁₅ are located in the extracellular portion of H2HR. The conserved residues of H2HR, C₁₀₀ and C₁₇₈, form a potential disulfide bridge and,
 10 D₁₂₄RYYAV₁₂₉, constitute a conserved G protein binding motif. The approximate locations of the TMS which define this molecule are: TMS1 contains the residues Q₃₀-Y₅₃; TMS2, T₇₀-R₉₀; TMS3, F₁₀₂-I₁₂₃; TMS4, A₁₄₄-S₁₆₈; TMS5, E₁₈₅-F₂₁₀; TMS6, T₂₇₂-A₂₉₅; and TMS7, S₃₀₅-G₃₂₆. The novel receptor has a W₁₉₂ substituting for the D₁₈₆ in TMS5; it has been suggested that the negatively charged residue in this position binds the positively charged imidazole
 15 ring of histamine. H2RH has two distinctive ATP binding motifs, S₂₉₃EALWGKS₃₀₀ and G₃₈₁GQPLGHS₃₈₈.

Figures 2A, 2B, 2C, 2D and 2E shows the chemical and structural homology among H2RH, (SEQ ID NO:1), canine H2R (GI 163952; SEQ ID NO:3), human H2R (GI 184088; SEQ ID NO:4), Cavia H2R (GI 791239; SEQ ID NO:5), rat H2R (GI 236184; SEQ ID
 20 NO:6), human alpha 1C adrenergic receptor (GI 927211; SEQ ID NO:7), and human 5-HT2B serotonin receptor (GI 475198; SEQ ID NO:8). H2RH shares approximately 41% identity with the most closely related canine H2R (GI 163952; SEQ ID NO:3). As illustrated by Figures 3 and 4, H2RH and canine H2R have rather typical T7G hydrophobicity plots and their calculated isoelectric points are 7.98 and 9.19, respectively. Northern analysis (not
 25 shown) shows that H2RH is expressed only in the bladder (BLADNOT06), spinal cord (SCORNOT03), colon (COLNNOT08), and endothelial cells of the coronary artery (ENDONOT01) libraries.

The invention also encompasses H2RH variants. A preferred H2RH variant is one having at least 80%, and more preferably 90%, amino acid sequence similarity to the H2RH
 30 amino acid sequence (SEQ ID NO:1). A most preferred H2RH variant is one having at least

95% amino acid sequence similarity to SEQ ID NO:1.

The invention also encompasses polynucleotides which encode H2RH. Accordingly, any nucleic acid sequence which encodes the amino acid sequence of H2RH can be used to generate recombinant molecules which express H2RH. In a particular embodiment, the invention encompasses the polynucleotide comprising the nucleic acid sequence of SEQ ID NO:2 as shown in Figures 1A, 1B, 1C and 1D.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of nucleotide sequences encoding H2RH, some bearing minimal homology to the nucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence of naturally occurring H2RH, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode H2RH and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring H2RH under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding H2RH or its derivatives possessing a substantially different codon usage. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding H2RH and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences, or portions thereof, which encode H2RH and its derivatives, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents that are well known in the art at the time of the filing of this application. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding H2RH or any portion thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed nucleotide sequences, and in particular, those shown in SEQ ID NO:2, under various conditions of stringency. Hybridization conditions are based on the melting temperature (T_m) of the nucleic acid binding complex or probe, as taught in Wahl, G.M. and S.L. Berger (1987; Methods Enzymol. 152:399-407) and Kimmel, A.R. (1987; Methods Enzymol. 152:507-511), and may be used at a defined stringency.

Altered nucleic acid sequences encoding H2RH which are encompassed by the invention include deletions, insertions, or substitutions of different nucleotides resulting in a polynucleotide that encodes the same or a functionally equivalent H2RH. The encoded protein may also contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent H2RH. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the biological activity of H2RH is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid; positively charged amino acids may include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; phenylalanine and tyrosine.

Also included within the scope of the present invention are alleles of the genes encoding H2RH. As used herein, an "allele" or "allelic sequence" is an alternative form of the gene which may result from at least one mutation in the nucleic acid sequence. Alleles may result in altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, one, or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

Methods for DNA sequencing which are well known and generally available in the art may be used to practice any embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase® (US Biochemical Corp, Cleveland, OH), Taq polymerase (Perkin Elmer), thermostable T7 polymerase (Amersham,

Chicago, IL), or combinations of recombinant polymerases and proofreading exonucleases such as the ELONGASE Amplification System marketed by Gibco BRL (Gaithersburg, MD). Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno, NV), Peltier Thermal Cycler (PTC200; MJ Research, Watertown, MA) and the ABI 377 DNA sequencers (Perkin Elmer).

The nucleic acid sequences encoding H2RH may be extended utilizing a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences such as promoters and regulatory elements. For example, one method which may be employed, "restriction-site" PCR, uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, G. (1993) PCR Methods Applic. 2:318-322). In particular, genomic DNA is first amplified in the presence of primer to linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR may also be used to amplify or extend sequences using divergent primers based on a known region (Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186). The primers may be designed using OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, MN), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68°-72° C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which may be used is capture PCR which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119). In this method, multiple restriction enzyme digestions and ligations may also be used to place an engineered double-stranded sequence into an unknown portion of the DNA molecule before performing PCR.

Another method which may be used to retrieve unknown sequences is that of Parker,

J.D. et al. (1991; Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PromoterFinder™ libraries to walk in genomic DNA (Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Also, random-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into the 5' and 3' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity may be converted to electrical signal using appropriate software (e.g. Genotyper™ and Sequence Navigator™, Perkin Elmer) and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode H2RH, or fusion proteins or functional equivalents thereof, may be used in recombinant DNA molecules to direct expression of H2RH in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and these sequences may be used to clone and express H2RH.

As will be understood by those of skill in the art, it may be advantageous to produce H2RH-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated

from the naturally occurring sequence.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter H2RH encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding H2RH may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of H2RH activity, it may be useful to encode a chimeric H2RH protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the H2RH encoding sequence and the heterologous protein sequence, so that H2RH may be cleaved and purified away from the heterologous moiety.

In another embodiment, sequences encoding H2RH may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M.H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223, Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 225-232). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of H2RH, or a portion thereof. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J.Y. et al. (1995) Science 269:202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer).

The newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography (e.g., Creighton, T. (1983) Proteins, Structures and Molecular Principles, WH Freeman and Co., New York, NY). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; Creighton, supra). Additionally, the amino acid sequence of H2RH, or any part thereof, may be altered during direct synthesis and/or combined using chemical

methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

In order to express a biologically active H2RH, the nucleotide sequences encoding H2RH or functional equivalents, may be inserted into appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence.

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding H2RH and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, NY, and Ausubel, F.M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY.

A variety of expression vector/host systems may be utilized to contain and express sequences encoding H2RH. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems.

The "control elements" or "regulatory sequences" are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the Bluescript® phagemid (Stratagene, LaJolla, CA) or pSport1™ plasmid (Gibco BRL) and the like may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO; and storage protein genes) or from plant

viruses (e.g., viral promoters or leader sequences) may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding H2RH, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for H2RH. For example, when large quantities of H2RH are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as Bluescript® (Stratagene), in which the sequence encoding H2RH may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β -galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors (Promega, Madison, WI) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast, *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel et al. (supra) and Grant et al. (1987) Methods Enzymol. 153:516-544.

In cases where plant expression vectors are used, the expression of sequences encoding H2RH may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ.

17:85-105). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, Hobbs, S. or Murry, L.E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, NY; pp. 191-196.

An insect system may also be used to express H2RH. For example, in one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The sequences encoding H2RH may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of H2RH will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, S. frugiperda cells or Trichoplusia larvae in which H2RH may be expressed (Engelhard, E.K. et al. (1994) Proc. Nat. Acad. Sci. 91:3224-3227).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding H2RH may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing H2RH in infected host cells (Logan, J. and Shenk, T. (1984) Proc. Natl. Acad. Sci. 81:3655-3659). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding H2RH. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding H2RH, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational

elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162).

5 In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding
10 and/or function. Different host cells such as CHO, HeLa, MDCK, HEK293, and WI38, which have specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is
15 preferred. For example, cell lines which stably express H2RH may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable
20 marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines.
25 These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. et al. (1977) Cell 11:223-32) and adenine phosphoribosyltransferase (Lowy, I. et al. (1980) Cell 22:817-23) genes which can be employed in tk⁻ or aprt⁻ cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler, M. et al. (1980) Proc. Natl.
30 Acad. Sci. 77:3567-70); npt, which confers resistance to the aminoglycosides neomycin and

G-418 (Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150:1-14) and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, supra). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-51). Recently, the use of visible markers has gained popularity with such markers as anthocyanins, β glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C.A. et al. (1995) Methods Mol. Biol. 55:121-131).

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequence encoding H2RH is inserted within a marker gene sequence, recombinant cells containing sequences encoding H2RH can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding H2RH under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells which contain the nucleic acid sequence encoding H2RH and express H2RH may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

The presence of polynucleotide sequences encoding H2RH can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or portions or fragments of polynucleotides encoding H2RH. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the sequences encoding H2RH to detect transformants containing DNA or RNA encoding H2RH. As used herein "oligonucleotides" or "oligomers" refer to a nucleic acid sequence of at least about 10 nucleotides and as many as about 60 nucleotides, preferably about 15 to 30 nucleotides, and more preferably about 20-25 nucleotides, which can be used as a probe or amplimer.

A variety of protocols for detecting and measuring the expression of H2RH, using either polyclonal or monoclonal antibodies specific for the protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay
5 utilizing monoclonal antibodies reactive to two non-interfering epitopes on H2RH is preferred, but a competitive binding assay may be employed. These and other assays are described, among other places, in Hampton, R. et al. (1990; Serological Methods, a Laboratory Manual, APS Press, St Paul, MN) and Maddox, D.E. et al. (1983; J. Exp. Med. 158:1211-1216).

10 A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding H2RH include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding H2RH, or any portions
15 thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits (Pharmacia & Upjohn, (Kalamazoo, MI); Promega (Madison WI); and U.S. Biochemical
20 Corp., Cleveland, OH). Suitable reporter molecules or labels, which may be used, include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding H2RH may be cultured under conditions suitable for the expression and recovery of the protein from cell culture.
25 The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode H2RH may be designed to contain signal sequences which direct secretion of H2RH through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join sequences
30 encoding H2RH to nucleotide sequence encoding a polypeptide domain which will facilitate

purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and H2RH may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing H2RH and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography as described in Porath, J. et al. (1992, Prot. Exp. Purif. 3:263-281) while the enterokinase cleavage site provides a means for purifying H2RH from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D.J. et al. (1993; DNA Cell Biol. 12:441-453).

In addition to recombinant production, fragments of H2RH may be produced by direct peptide synthesis using solid-phase techniques Merrifield J. (1963) J. Am. Chem. Soc. 85:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Various fragments of H2RH may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

THERAPEUTICS

In another embodiment of the invention, H2RH may be used to screen drug libraries for antagonists which may be used therapeutically to block or modulate the activity of the receptor. Antagonists of H2RH may be used in any of those transient or chronic situations where inhibition is desirable. Such antagonists or inhibitors may be produced using methods which are generally known in the art, and include particularly the use of purified H2RH to produce antibodies or to screen libraries of pharmaceutical agents for those which specifically bind and inhibit the activities of H2RH.

For example, in one aspect, antagonists which specifically bind H2RH may be

administered to the subject's gastric mucosa in conjunction with a suitable pharmaceutical carrier. Control of H2RH expression and activity may be used to treat duodenal ulcers and may be especially useful in combination with other therapeutic agents, such as the antacid and antibiotic regimes used to eliminate Helicobacter pylori and cure peptic ulcers. Reducing the amount of gastric acids secreted at mealtime as well as between meals will provide the time and a noncorrosive environment in which cells of the gastric mucosa can heal. In some cases, combinations of therapeutic agents having different cellular mechanisms of action and potentially synergistic effects will allow the use of lower effective doses of each agent with lessening side effects.

In another embodiment, antagonists of H2RH may be administered to the lungs of a subject in those cases in which the hyperactivity of H2RH is triggering allergic or asthmatic responses. In both responses, the ability to modulate H2HR activity may prevent the degranulation of excess numbers of monocytes, macrophages and other immune system cells that release the enzymes which produce unwarranted tissue destruction. Such therapy may also be warranted in the treatment of allergies, asthma, bronchitis, emphysema, aspergillosis, tuberculosis, and the like.

In another embodiment, antagonists of H2RH may be administered to a subject for the modulation of muscle spasms and cramping associated with gastric distress including but not limited to gastritis, flu, colitis, Crohn's disease and the like. Antagonists could also be employed to prevent the disorientation associated with motion sickness as well as the undue agitation associated with various conditions of the central nervous system including, but not limited to, diseases such as Alzheimer's disease, ataxia, Eaton-Lambert syndrome, epilepsy, myasthenia gravis, Parkinson's disease, and the like, or to conditions caused by the compression of brain or spinal tissues caused by tumors.

Since the novel H2RH was discovered in bladder and has a strong association with endothelial/epithelial tissues, antagonists may be administered to a subject to treat infections or inflammation of the urinary tract and bladder, and may administered to women with pelvic inflammatory disease with the intent of preventing infertility. In another embodiment, antagonists may be used to modulate H2RH in endothelial cells of the cardiovascular system and treat diseases such as arteriosclerosis, cardiomyopathy, endocarditis, ischemia, and the

like.

The antibodies may be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies, (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others, may be immunized by injection with H2RH or any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the peptides, fragments, or oligopeptides used to induce antibodies to H2RH have an amino acid sequence consisting of at least five amino acids, and more preferably at least 10 amino acids. It is also preferable that they are identical to a portion of the amino acid sequence of the natural protein, and they may contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of H2RH amino acids may be fused with those of another protein such as keyhole limpet hemocyanin and antibody produced against the chimeric molecule.

Monoclonal antibodies to H2RH may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. 80:2026-2030; Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120).

In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison, S.L. et al.

(1984) Proc. Natl. Acad. Sci. 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; Takeda, S. et al. (1985) Nature 314:452-454). Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce H2RH-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries (Burton D.R. (1991) Proc. Natl. Acad. Sci. 88:11120-3).

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. 86: 3833-3837; Winter, G. et al. (1991) Nature 349:293-299).

Antibody fragments which contain specific binding sites for H2RH may also be generated. For example, such fragments include, but are not limited to, the F(ab')₂ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse, W.D. et al. (1989) Science 254:1275-1281).

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between H2RH and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering H2RH epitopes is preferred, but a competitive binding assay may also be employed (Maddox, supra).

In another embodiment of the invention, the polynucleotides encoding H2RH, or any fragment thereof, or antisense molecules, may be used for therapeutic purposes. In one aspect, antisense to the polynucleotide encoding H2RH may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding H2RH. Thus,

antisense molecules may be used to modulate H2RH activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligomers or larger fragments, can be designed from various locations along the coding or control regions of sequences encoding H2RH.

5 Expression vectors derived from retroviruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids may be used for delivery of nucleotide sequences to the targeted organ, tissue or cell population. Methods which are well known to those skilled in the art can be used to construct recombinant vectors which will express antisense molecules complementary to the polynucleotides of the gene encoding H2RH. These techniques are
10 described both in Sambrook et al. (supra) and in Ausubel et al. (supra).

 Genes encoding H2RH can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide or fragment thereof which encodes H2RH. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may
15 continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector and even longer if appropriate replication elements are part of the vector system.

 As mentioned above, modifications of gene expression can be obtained by designing antisense molecules, DNA, RNA, or PNA, to the control regions of the gene encoding H2RH,
20 i.e., the promoters, enhancers, and introns. Oligonucleotides derived from the transcription initiation site, e.g., between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules.
25 Recent therapeutic advances using triplex DNA have been described in the literature (Gee, J.E. et al. (1994) In: Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY). The antisense molecules may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

 Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific
30 cleavage of RNA. The mechanism of ribozyme action involves sequence-specific

hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples which may be used include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding H2RH.

5 Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the
10 oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

 Antisense molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for
15 chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding H2RH. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize antisense RNA constitutively or
20 inducibly can be introduced into cell lines, cells, or tissues.

 RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in
25 the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

 Many methods for introducing vectors into cells or tissues are available and equally
30 suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced

into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection and by liposome injections may be achieved using methods which are well known in the art.

Any of the therapeutic methods described above may be applied to any suitable subject including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

An additional embodiment of the invention relates to the administration of a pharmaceutical composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of H2RH, antibodies to H2RH, mimetics, agonists, antagonists, or inhibitors of H2RH. The compositions may be administered alone or in combination with at least one other agent, such as stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the

preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

5 The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

10 The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

15 After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of H2RH, such labeling would include amount, frequency, and method of administration.

20 Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

25 For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

30 A therapeutically effective dose refers to that amount of active ingredient, for example H2RH or fragments thereof, antibodies of H2RH, agonists, antagonists or inhibitors of H2RH, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50

(the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50.

Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind H2RH may be used for the diagnosis of conditions or diseases characterized by expression of H2RH, or in assays to monitor patients being treated with H2RH, agonists, antagonists or inhibitors. The antibodies useful for diagnostic purposes may be prepared in the same manner as those described above for therapeutics. Diagnostic assays for H2RH include methods which utilize the antibody and a label to detect H2RH in human body fluids or extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by joining them, either

covalently or non-covalently, with a reporter molecule. A wide variety of reporter molecules which are known in the art may be used, several of which are described above.

A variety of protocols including ELISA, RIA, and FACS for measuring H2RH are known in the art and provide a basis for diagnosing altered or abnormal levels of H2RH expression. Normal or standard values for H2RH expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to H2RH under conditions suitable for complex formation. The amount of standard complex formation may be quantified by various methods, but preferably by photometric, means. Quantities of H2RH expressed in subject, control and disease, samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding H2RH may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, antisense RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of H2RH may be correlated with disease. The diagnostic assay may be used to distinguish between absence, presence, and excess expression of H2RH, and to monitor regulation of H2RH levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding H2RH or closely related molecules, may be used to identify nucleic acid sequences which encode H2RH. The specificity of the probe, whether it is made from a highly specific region, e.g., 10 unique nucleotides in the 5' regulatory region, or a less specific region, e.g., especially in the 3' coding region, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low) will determine whether the probe identifies only naturally occurring sequences encoding H2RH, alleles, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably contain at least 50% of the nucleotides from any of the H2RH encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and derived from the nucleotide sequence of SEQ ID NO:2 or from genomic sequence including promoter,

enhancer elements, and introns of the naturally occurring H2RH.

Means for producing specific hybridization probes for DNAs encoding H2RH include the cloning of nucleic acid sequences encoding H2RH or H2RH derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, radionuclides such as ³²P or ³⁵S, or enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding H2RH may be used for the diagnosis of any condition or disease which is associated with the expression and activity of H2RH. Examples of such conditions include, but are not limited to damage, disorders or diseases of the cardiovascular, digestive, immune, respiratory, reproductive, urinary or central nervous systems. The level at which H2RH is expressed or active in the endothelium of an artery or vein may correlate with the immune system interactions in arteriosclerosis and present an opportunity to diagnose those events. Similarly endothelial/epithelial and immune cell interactions may be monitored in the lung, bladder, or gastric mucosa as they correlate with a conditions or diseases affecting those structures.

The polynucleotide sequences encoding H2RH may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; or in dip stick, pin, ELISA or chip assays utilizing fluids or tissues from patient biopsies to detect altered H2RH expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding H2RH may be useful in assays that detect activation or induction of various cancers, particularly those mentioned above. The nucleotide sequences encoding H2RH may be labeled by standard methods, and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the biopsied or extracted sample is significantly altered from that of a comparable control sample, the nucleotide sequences have hybridized with nucleotide sequences in the sample, and the

presence of altered levels of nucleotide sequences encoding H2RH in the sample indicates the presence of the associated disease. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or in monitoring the treatment of an individual patient.

5 In order to provide a basis for the diagnosis of disease associated with expression of H2RH, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, which encodes H2RH, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the
10 values obtained from normal subjects with those from an experiment where a known amount of a substantially purified polynucleotide is used. Standard values obtained from normal samples may be compared with values obtained from samples from patients who are symptomatic for disease. Deviation between standard and subject values is used to establish the presence of disease.

15 Once disease is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to evaluate whether the level of expression in the patient begins to approximate that which is observed in the normal patient. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

20 With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the
25 development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding H2RH may involve the use of PCR. Such oligomers may be chemically synthesized, generated enzymatically, or produced from a recombinant source. Oligomers will preferably consist of two nucleotide sequences, one with sense orientation (5'→3') and another with
30 antisense (3'←5'), employed under optimized conditions for identification of a specific gene

or condition. The same two oligomers, nested sets of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for detection and/or quantitation of closely related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of H2RH include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and standard curves onto which the experimental results are interpolated (Melby, P.C. et al. (1993) J. Immunol. Methods, 159:235-244; Duplaa, C. et al. (1993) Anal. Biochem. 229-236). The speed of quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In another embodiment of the invention, the nucleic acid sequences which encode H2RH may also be used to generate hybridization probes which are useful for mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome or to a specific region of the chromosome using well known techniques. Such techniques include FISH, FACS, or artificial chromosome constructions, such as yeast artificial chromosomes, bacterial artificial chromosomes, bacterial P1 constructions or single chromosome cDNA libraries as reviewed in Price, C.M. (1993) Blood Rev. 7:127-134, and Trask, B.J. (1991) Trends Genet. 7:149-154.

FISH (as described in Verma et al. (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, NY) may be correlated with other physical chromosome mapping techniques and genetic map data. Examples of genetic map data can be found in the 1994 Genome Issue of Science (265:1981f). Correlation between the location of the gene encoding H2RH on a physical chromosomal map and a specific disease, or predisposition to a specific disease, may help delimit the region of DNA associated with that genetic disease. The nucleotide sequences of the subject invention may be used to detect differences in gene sequences between normal, carrier, or affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques such as linkage analysis using established chromosomal markers may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a

particular human chromosome is not known. New sequences can be assigned to chromosomal arms, or parts thereof, by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, for example, AT to 11q22-23 (Gatti, R.A. et al. (1988) Nature 336:577-580), any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc. among normal, carrier, or affected individuals.

In another embodiment of the invention, H2RH, its catalytic or immunogenic fragments or oligopeptides thereof, can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes, between H2RH and the agent being tested, may be measured.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the protein of interest as described in published PCT application WO84/03564. In this method, as applied to H2RH large numbers of different small test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with H2RH, or fragments thereof, and washed. Bound H2RH is then detected by methods well known in the art. Purified H2RH can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding H2RH specifically compete with a test compound for binding H2RH. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with H2RH.

In additional embodiments, the nucleotide sequences which encode H2RH may be used in any molecular biology techniques that have yet to be developed, provided the new

techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

The examples below are provided to illustrate the subject invention and are not included for the purpose of limiting the invention.

INDUSTRIAL APPLICABILITY

I BLADNOT06 cDNA Library Construction

The BLADNOT06 cDNA library was constructed from microscopically normal bladder tissue obtained from a 66-year-old Caucasian male (specimen #0320A; Mayo Clinic, Rochester, MN). The normal and tumorous tissues were excised during a radical cystectomy for transitional cell carcinoma of the bladder. At the time of surgery, the patient was taking Dyazide® (SmithKline Beecham, Philadelphia, PA) and iron. Patient history included prostatic inflammatory disease, malignant neoplasm of the prostate, a transurethral prostatectomy, urinary diversion to the bowel, tobacco abuse, a lung neoplasm, and hypertension.

The frozen tissue was homogenized and lysed using a Brinkmann Homogenizer Polytron PT-3000 (Brinkmann Instruments, Westbury, NJ) in guanidinium isothiocyanate solution. The lysate was centrifuged over a 5.7 M CsCl cushion using an Beckman SW28 rotor in a Beckman L8-70M Ultracentrifuge (Beckman Instruments) for 18 hours at 25,000 rpm at ambient temperature. The RNA was extracted with acid phenol pH 4.7, precipitated using 0.3 M sodium acetate and 2.5 volumes of ethanol, resuspended in RNase-free water, and DNase treated at 37°C. The RNA extraction was repeated with acid phenol pH 4.7 and precipitated with sodium acetate and ethanol as before. The mRNA was then isolated using the Qiagen Oligotex kit (QIAGEN; Chatsworth, CA) and used to construct the cDNA library.

The mRNA was handled according to the recommended protocols in the SuperScript Plasmid System for cDNA Synthesis and Plasmid Cloning (Cat. #18248-013; Gibco/BRL). The commercial plasmid pSPORT 1 (Gibco/BRL) was digested with EcoR I restriction enzyme (New England Biolabs, Beverly, MA). The overhanging ends of the plasmid were filled in using Klenow enzyme (New England Biolabs) and 2'-deoxynucleotide 5'-triphosphates (dNTPs). The plasmid was self-ligated and transformed into the bacterial host, *E. coli* strain JM 109. An intermediate plasmid produced by the bacteria failed to digest

with EcoR I confirming the desired loss of the EcoR I restriction site.

This intermediate plasmid (pSPORT 1- Δ RI) was then digested with Hind III restriction enzyme (New England Biolabs) and the overhang was filled in with Klenow and dNTPs. A 10-mer linker of sequence 5'...CGGAATTCCG...3' was phosphorylated and ligated onto the blunt ends. The product of the ligation reaction was digested with EcoR I and self-ligated. Following transformation into JM109 host cells, plasmids were isolated and screened for the digestibility with EcoR I but not with Hind III. A single colony which met this criteria was designated pINCY 1. The plasmid produced by this colony was sequenced and found to contain several copies of the 10-mer linker. These extra linkers did not present a problem as they were eliminated when the vector was prepared for cloning.

The plasmid was tested for its ability to incorporate cDNAs from a library prepared using Not I and EcoR I restriction enzymes. Several clones were sequenced and a single clone containing an insert of approximately 0.8 kb was selected to prepare a large quantity of the plasmid for library production. After digestion with Not I and EcoR I, the plasmid and the cDNA insert were isolated on an agarose gel and the vector was purified on a QIAQuick™ (QIAGEN, Chatsworth, CA) column for use in library construction.

cDNAs were fractionated on a Sepharose CL4B column (Cat. #275105-01; Pharmacia), and those cDNAs exceeding 400 bp were ligated into pSport I. The plasmid pSport I was subsequently transformed into DH5a™ competent cells (Cat. #18258-012; Gibco/BRL).

II Isolation and Sequencing of cDNA Clones

Plasmid DNA was released from the cells and purified using the REAL Prep 96 Plasmid Kit for Rapid Extraction Alkaline Lysis Plasmid Minipreps (Catalog #26173; QIAGEN, Inc.). This kit enabled the simultaneous purification of 96 samples in a 96-well block using multi-channel reagent dispensers. The recommended protocol was employed except for the following changes: 1) the bacteria were cultured in 1 ml of sterile Terrific Broth (Catalog #22711, LIFE TECHNOLOGIES™) with carbenicillin at 25 mg/L and glycerol at 0.4%; 2) after inoculation, the cultures were incubated for 19 hours and at the end of incubation, the cells were lysed with 0.3 ml of lysis buffer; and 3) following isopropanol precipitation, the plasmid DNA pellet was resuspended in 0.1 ml of distilled water. After the

last step in the protocol, samples were transferred to a 96-well block for storage at 4° C.

The cDNAs were sequenced by the method of Sanger et al. (1975, J. Mol. Biol. 94:441f), using a Hamilton Micro Lab 2200 (Hamilton, Reno, NV) in combination with Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown, MA) and Applied Biosystems 377 DNA Sequencing Systems.

Most of the sequences disclosed herein were sequenced according to standard ABI protocols, using ABI kits (Cat. Nos. 79345, 79339, 79340, 79357, 79355). The solution volumes were used at 0.25x - 1.0x concentrations. Some of the sequences disclosed herein were sequenced using different solutions and dyes which, unless otherwise noted, came from Amersham Life Science (Cleveland, OH).

First, stock solutions were prepared with HPLC water. The following solutions were each mixed by vortexing for 2 min: 1) Tris-EDTA (TE) Buffer was prepared by adding 49 ml water to 1 ml 50x Tris-EDTA concentrate, and 2) 10% Reaction Buffer was prepared by adding 45 ml water to 5 ml Concentrated Thermo Sequenase (TS) Reaction Buffer.

Second, 0.2 μ M energy transfer (ET) primers were prepared in the following manner. Each primer tube was centrifuged prior to opening to assure that all primer powder was on the bottom of the tube. After each solubilization step, the mixture was vortexed for 2 min and then centrifuged for about 10 sec in a table-top centrifuge. 1 ml of 1x TE was added to each primer powder; adenine and cytosine dissolved primers (5-carboxyrhodamine-6G (R6G) and 6-carboxyfluorescein (FAM), respectively), were diluted with 9 ml 1x TE. Guanine and thymine dyes (*N,N,N',N''*-tetramethyl-6-carboxyrhodamine (TAM) and 6-carboxy-X-rhodamine (ROX), respectively) were diluted with 19 ml 1x TE.

Next, the sequencing reaction ready mix was prepared as follows: 1) nucleotides A and C (8 ml of each) were added to 6 ml ET primer and 18 ml TS reaction buffer; and 2) nucleotides G and T (8 ml of each) were added to 6 ml ET primer and 18 ml TS reaction buffer.

After vortexing for 2 min and centrifuging for 20 sec, the resulting solution was divided into tubes in volumes of 8 ml per tube in order to make 1x (A,C) and 2x (G,T) solutions.

Prior to thermal cycling, each nucleotide was individually mixed with DNA template

in the following proportions:

Reagent	A(μ L)	C(μ L)	G(μ L)	T(μ L)	
Reaction ready premix		2	2	4	4
DNA template	1	1	2		2
Total Volume	3	3	6	6	

These solutions undergo the usual thermal cycling:

1. Rapid thermal ramp to 94° C (94° C for 20 sec)*
2. Rapid thermal ramp to 50° C (50° C for 40 sec)*
3. Rapid thermal ramp to 68° C (68° C for 60 sec)*
- * Steps 1, 2, and 3 were repeated for 15 cycles
4. Rapid thermal ramp to 94° C (94° C for 20 sec)**
5. Rapid thermal ramp to 68° C (68° C for 60 sec)**
- ** Steps 4 and 5 were repeated for 15 cycles
6. Rapid thermal ramp to 4° C and hold until ready to combine.

After thermal cycling, the A, C, G, and T reactions with each DNA template were combined. Then, 50 μ L 100% ethanol was added and the solution was spun at 4° C for 30 min. The supernatant was decanted and the pellet was rinsed with 100 μ L 70% ethanol.

After being spun for 15 min the supernatant was discarded and the pellet was dried for 15 min under vacuum. The DNA sample was dissolved in 3 μ L of formamide/50 mM EDTA. The resulting samples were loaded on wells in volumes of 2 μ L per well for sequencing in ABI sequencers.

III Homology Searching of cDNA Clones and Their Deduced Proteins

Each cDNA was compared to sequences in GenBank using a search algorithm developed by Applied Biosystems and incorporated into the INHERIT™ 670 sequence analysis system. In this algorithm, Pattern Specification Language (TRW Inc, Los Angeles, CA) was used to determine regions of homology. The three parameters that determine how the sequence comparisons run were window size, window offset, and error tolerance. Using a combination of these three parameters, the DNA database was searched for sequences containing regions of homology to the query sequence, and the appropriate sequences were scored with an initial value. Subsequently, these homologous regions were examined using dot matrix homology plots to distinguish regions of homology from chance matches.

Smith-Waterman alignments were used to display the results of the homology search.

Peptide and protein sequence homologies were ascertained using the INHERIT- 670 sequence analysis system using the methods similar to those used in DNA sequence homologies. Pattern Specification Language and parameter windows were used to search
5 protein databases for sequences containing regions of homology which were scored with an initial value. Dot-matrix homology plots were examined to distinguish regions of significant homology from chance matches.

BLAST, which stands for Basic Local Alignment Search Tool (Altschul, S.F. (1993) J. Mol. Evol. 36:290-300; Altschul et al. (1990) J. Mol. Biol. 215:403-410), was used to
10 search for local sequence alignments. BLAST produces alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologs. BLAST is useful for matches which do not contain gaps. The fundamental unit of BLAST algorithm output is the High-scoring Segment Pair (HSP).

15 An HSP consists of two sequence fragments of arbitrary but equal lengths whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cutoff score set by the user. The BLAST approach is to look for HSPs between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of
20 significance. The parameter E establishes the statistically significant threshold for reporting database sequence matches. E is interpreted as the upper bound of the expected frequency of chance occurrence of an HSP (or set of HSPs) within the context of the entire database search. Any database sequence whose match satisfies E is reported in the program output.

IV Northern Analysis

25 Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound (Sambrook et al., supra).

Analogous computer techniques using BLAST (Altschul, S.F. 1993 and 1990, supra) are used to search for identical or related molecules in nucleotide databases such as GenBank
30 or the LIFESEQ™ database (Incyte Pharmaceuticals). This analysis is much faster than

multiple, membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or homologous.

The basis of the search is the product score which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1-2% error; and at 70, the match will be exact. Homologous molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

The results of northern analysis are reported as a list of libraries in which the transcript encoding H2RH occurs. Abundance and percent abundance are also reported. Abundance directly reflects the number of times a particular transcript is represented in a cDNA library, and percent abundance is abundance divided by the total number of sequences examined in the cDNA library.

V Extension of Polynucleotides Encoding H2RH to Full Length or to Recover Regulatory Sequences

Full length nucleic acid sequences encoding H2RH (SEQ ID NO:2) are used to design oligonucleotide primers for extending a partial nucleotide sequence to full length or for obtaining 5' or 3', intron or other control sequences from genomic libraries. One primer is synthesized to initiate extension in the antisense direction (XLR) and the other is synthesized to extend sequence in the sense direction (XLF). Primers are used to facilitate the extension of the known sequence "outward" generating amplicons containing new, unknown nucleotide sequence for the region of interest. The initial primers are designed from the cDNA using OLIGO 4.06 (National Biosciences), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68°-72° C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations is avoided.

The original, selected cDNA libraries, or a human genomic library are used to extend

the sequence; the latter is most useful to obtain 5' upstream regions. If more extension is necessary or desired, additional sets of primers are designed to further extend the known region.

By following the instructions for the XL-PCR kit (Perkin Elmer) and thoroughly mixing the enzyme and reaction mix, high fidelity amplification is obtained. Beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit, PCR is performed using the Peltier Thermal Cycler (PTC200; M.J. Research, Watertown, MA) and the following parameters:

10	Step 1	94° C for 1 min (initial denaturation)
	Step 2	65° C for 1 min
	Step 3	68° C for 6 min
	Step 4	94° C for 15 sec
	Step 5	65° C for 1 min
	Step 6	68° C for 7 min
15	Step 7	Repeat step 4-6 for 15 additional cycles
	Step 8	94° C for 15 sec
	Step 9	65° C for 1 min
	Step 10	68° C for 7:15 min
	Step 11	Repeat step 8-10 for 12 cycles
20	Step 12	72° C for 8 min
	Step 13	4° C (and holding)

A 5-10 μ l aliquot of the reaction mixture is analyzed by electrophoresis on a low concentration (about 0.6-0.8%) agarose mini-gel to determine which reactions were successful in extending the sequence. Bands thought to contain the largest products are selected and removed from the gel. Further purification involves using a commercial gel extraction method such as QIAQuick™ (QIAGEN Inc., Chatsworth, CA). After recovery of the DNA, Klenow enzyme is used to trim single-stranded, nucleotide overhangs creating blunt ends which facilitate religation and cloning.

After ethanol precipitation, the products are redissolved in 13 μ l of ligation buffer, 1 μ l T4-DNA ligase (15 units) and 1 μ l T4 polynucleotide kinase are added, and the mixture is incubated at room temperature for 2-3 hours or overnight at 16° C. Competent *E. coli* cells (in 40 μ l of appropriate media) are transformed with 3 μ l of ligation mixture and cultured in 80 μ l of SOC medium (Sambrook et al., supra). After incubation for one hour at 37° C, the

whole transformation mixture is plated on Luria Bertani (LB)-agar (Sambrook et al., supra) containing 2x Carb. The following day, several colonies are randomly picked from each plate and cultured in 150 μ l of liquid LB/2x Carb medium placed in an individual well of an appropriate, commercially-available, sterile 96-well microtiter plate. The following day, 5 μ l of each overnight culture is transferred into a non-sterile 96-well plate and after dilution 1:10 with water, 5 μ l of each sample is transferred into a PCR array.

For PCR amplification, 18 μ l of concentrated PCR reaction mix (3.3x) containing 4 units of rTth DNA polymerase, a vector primer, and one or both of the gene specific primers used for the extension reaction are added to each well. Amplification is performed using the following conditions:

- | | |
|--------|--|
| Step 1 | 94° C for 60 sec |
| Step 2 | 94° C for 20 sec |
| Step 3 | 55° C for 30 sec |
| Step 4 | 72° C for 90 sec |
| Step 5 | Repeat steps 2-4 for an additional 29 cycles |
| Step 6 | 72° C for 180 sec |
| Step 7 | 4° C (and holding) |

Aliquots of the PCR reactions are run on agarose gels together with molecular weight markers. The sizes of the PCR products are compared to the original partial cDNAs, and appropriate clones are selected, ligated into plasmid, and sequenced.

VI Labeling and Use of Hybridization Probes

Hybridization probes derived from SEQ ID NO:2 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base-pairs, is specifically described, essentially the same procedure is used with larger cDNA fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 (National Biosciences), labeled by combining 50 pmol of each oligomer and 250 μ Ci of [γ -³²P] adenosine triphosphate (Amersham) and T4 polynucleotide kinase (DuPont NEN®, Boston, MA). The labeled oligonucleotides are substantially purified with Sephadex G-25 superfine resin column (Pharmacia & Upjohn). A portion containing 10⁷ counts per minute of each of the sense and antisense oligonucleotides is used in a typical membrane based hybridization analysis of human genomic DNA digested with one of the following endonucleases (Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II; DuPont NEN®).

The DNA from each digest is fractionated on a 0.7 percent agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham, NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT AR™ film (Kodak, Rochester, NY) is exposed to the blots in a Phosphorimager cassette (Molecular Dynamics, Sunnyvale, CA) for several hours, hybridization patterns are compared visually.

VII Antisense Molecules

Antisense molecules to the H2RH-encoding sequence, or any part thereof, is used to inhibit in vivo or in vitro expression of naturally occurring H2RH. Although use of antisense oligonucleotides, comprising about 20 base-pairs, is specifically described, essentially the same procedure is used with larger cDNA fragments. An oligonucleotide based on the coding sequences of H2RH, as shown in Figures 1A, 1B, 1C and 1D, is used to inhibit expression of naturally occurring H2RH. The complementary oligonucleotide is designed from the most unique 5' sequence as shown in Figures 1A, 1B, 1C and 1D and used either to inhibit transcription by preventing promoter binding to the upstream nontranslated sequence or translation of an H2RH-encoding transcript by preventing the ribosome from binding. Using an appropriate portion of the signal and 5' sequence of SEQ ID NO:2, an effective antisense oligonucleotide includes any 15-20 nucleotides spanning the region which translates into the signal or 5' coding sequence of the polypeptide as shown in Figures 1A, 1B, 1C and 1D.

VIII Expression of H2RH

Expression of H2RH is accomplished by subcloning the cDNAs into appropriate vectors and transforming the vectors into host cells. In this case, the cloning vector, pSport, previously used for the generation of the cDNA library is used to express H2RH in E. coli. Upstream of the cloning site, this vector contains a promoter for β -galactosidase, followed by sequence containing the amino-terminal Met, and the subsequent seven residues of β -galactosidase. Immediately following these eight residues is a bacteriophage promoter useful for transcription and a linker containing a number of unique restriction sites.

Induction of an isolated, transformed bacterial strain with IPTG using standard methods produces a fusion protein which consists of the first eight residues of

β -galactosidase, about 5 to 15 residues of linker, and the full length protein. The signal residues direct the secretion of H2RH into the bacterial growth media which can be used directly in the following assay for activity.

IX Demonstration of H2RH Activity

5 Functional, cloned T7Gs may be expressed in heterologous expression systems and their biological activity assessed. One procedure for testing chimeric receptors is based on the procedure utilizing the P_{2U} purinergic receptor (P_{2U}) as published by Erb, L. et al. (1993; Proc. Natl. Acad. Sci. 90:10449-53). Function of the chimeric receptor can easily be tested in cultured K562 human leukemia cells because these cells lack P_{2U} receptors. K562 cells are
10 transfected with expression vectors containing either normal or chimeric P_{2U} and loaded with fura-a, fluorescent probe for Ca^{++} . Activation of properly assembled and functional extracellular H2RH-transmembrane/intracellular P_{2U} receptors with extracellular UTP or ATP mobilizes intracellular Ca^{++} which reacts with fura-a and is measured spectrofluorometrically. Bathing the transfected K562 cells in microwells containing appropriate ligands will trigger
15 binding and fluorescent activity defining effectors of H2RH. Once ligand and function are established, the P_{2U} system is useful for defining antagonists or inhibitors which block binding and prevent such fluorescent reactions.

An alternative procedure for testing H2RH activity utilizes the baculovirus expression system in Sf9 insect cells. Insect cells are the perfect model system because no T7Gs have
20 been identified in insects and G proteins are expressed at low levels. Additionally, Sf9 cells are capable of both the co-translational and post-translational processing events which facilitate receptor and G protein subunit interaction (Taussig, R. et al. (1994) Methods Enzymol. 238:95-108). Infection of the Sf9 cells with recombinant baculovirus containing H2RH results in cells with H2RH properly expressed and positioned in the cell membrane.
25 Agonists and antagonists which specifically bind the receptor can be identified and measured using techniques well known in the art.

X Production of H2RH Specific Antibodies

H2RH that is substantially purified using PAGE electrophoresis (Sambrook, supra), or other purification techniques, is used to immunize rabbits and to produce antibodies using
30 standard protocols. The amino acid sequence deduced from SEQ ID NO:2 is analyzed using

DNASTAR software (DNASTAR Inc) to determine regions of high immunogenicity and a corresponding oligopolypeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions, is described by Ausubel et al. (supra), and others.

5 Typically, the oligopeptides are 15 residues in length, synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry, and coupled to keyhole limpet hemocyanin (KLH, Sigma, St. Louis, MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS; Ausubel et al., supra). Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested
10 for anti-peptide activity, for example, by binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radioiodinated, goat anti-rabbit IgG.

XI Purification of Naturally Occurring H2RH Using Specific Antibodies

Naturally occurring or recombinant H2RH is substantially purified by immunoaffinity
15 chromatography using antibodies specific for H2RH. An immunoaffinity column is constructed by covalently coupling H2RH antibody to an activated chromatographic resin, such as CnBr-activated Sepharose (Pharmacia & Upjohn). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing H2RH is passed over the immunoaffinity column, and the column is
20 washed under conditions that allow the preferential absorbance of H2RH (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/H2RH binding (eg, a buffer of pH 2-3 or a high concentration of a chaotrope, such as urea or thiocyanate ion), and H2RH is collected.

XII Identification of Molecules Which Interact with H2RH

25 H2RH or biologically active fragments thereof are labeled with ¹²⁵I Bolton-Hunter reagent (Bolton, A.E. and W.M. Hunter (1973) Biochem. J. 133: 529-38). Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled H2RH, washed and any wells with labeled H2RH complex are assayed. Data obtained using different concentrations of H2RH are used to calculate values for the number, affinity, and
30 association of H2RH with the candidate molecules.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

- (i) APPLICANT: INCYTE PHARMACEUTICALS, INC.
- (ii) TITLE OF THE INVENTION: NOVEL HISTAMINE H2 RECEPTOR
- (iii) NUMBER OF SEQUENCES: 8
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: INCYTE PHARMACEUTICALS, INC.
 - (B) STREET: 3174 Porter Drive
 - (C) CITY: Palo Alto
 - (D) STATE: CA
 - (E) COUNTRY: US
 - (F) ZIP: 94304
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Diskette
 - (B) COMPUTER: IBM Compatible
 - (C) OPERATING SYSTEM: DOS
 - (D) SOFTWARE: FastSEQ Version 2.0
- (vi) CURRENT APPLICATION DATA:
 - (A) PCT APPLICATION NUMBER: To Be Assigned
 - (B) FILING DATE: Herewith
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/748,485
 - (B) FILING DATE: 7-NOV-1996
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Billings, Lucy J.
 - (B) REGISTRATION NUMBER: 36,749
 - (C) REFERENCE/DOCKET NUMBER: PF-0159 PCT
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 650-855-0555
 - (B) TELEFAX: 650-845-4166
 - (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 454 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (vii) IMMEDIATE SOURCE:
 (A) LIBRARY: Consensus
 (B) CLONE: 1722180

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Ser Leu Asn Ser Ser Leu Ser Cys Arg Lys Glu Leu Ser Asn Leu
1 5 10 15

Thr Glu Gly Glu Gly Gly Glu Gly Gly Val Ile Ile Thr Gln Phe Ile
 20 25 30
 Ala Ile Ile Val Ile Thr Ile Phe Val Cys Leu Gly Asn Leu Val Ile
 35 40 45
 Val Val Thr Leu Tyr Lys Lys Ser Tyr Leu Leu Thr Leu Ser Asn Lys
 50 55 60
 Phe Val Phe Ser Leu Thr Leu Ser Asn Phe Leu Leu Ser Val Leu Val
 65 70 75 80
 Leu Pro Phe Val Val Thr Ser Ser Ile Arg Arg Glu Trp Ile Phe Gly
 85 90 95
 Val Val Trp Cys Asn Phe Ser Ala Leu Leu Tyr Leu Leu Ile Ser Ser
 100 105 110
 Ala Ser Met Leu Thr Leu Gly Val Ile Ala Ile Asp Arg Tyr Tyr Ala
 115 120 125
 Val Leu Tyr Pro Met Val Tyr Pro Met Lys Ile Thr Gly Asn Arg Ala
 130 135 140
 Val Met Ala Leu Val Tyr Ile Trp Leu His Ser Leu Ile Gly Cys Leu
 145 150 155 160
 Pro Pro Leu Phe Gly Trp Ser Ser Val Glu Phe Asp Glu Phe Lys Trp
 165 170 175
 Met Cys Val Ala Ala Trp His Arg Glu Pro Gly Tyr Thr Ala Phe Trp
 180 185 190
 Gln Ile Trp Cys Ala Leu Phe Pro Phe Leu Val Met Leu Val Cys Tyr
 195 200 205
 Gly Phe Ile Phe Arg Val Ala Arg Val Lys Ala Arg Lys Val His Cys
 210 215 220
 Gly Thr Val Val Ile Val Glu Glu Asp Ala Gln Arg Thr Gly Val Arg
 225 230 235 240
 Lys Asn Ser Ser Thr Ser Thr Ser Ser Ser Gly Ser Arg Arg Asn Ala
 245 250 255
 Phe Gln Gly Val Val Tyr Ser Ala Asn Gln Cys Lys Ala Leu Ile Thr
 260 265 270
 Ile Leu Val Val Leu Gly Ala Phe Met Val Thr Trp Gly Pro Tyr Met
 275 280 285
 Val Val Ile Ala Ser Glu Ala Leu Trp Gly Lys Ser Ser Val Ser Pro
 290 295 300
 Ser Leu Glu Thr Trp Ala Thr Trp Leu Ser Phe Ala Ser Ala Val Cys
 305 310 315 320
 His Pro Leu Ile Tyr Gly Leu Trp Asn Lys Thr Val Arg Lys Glu Leu
 325 330 335
 Leu Gly Met Cys Phe Gly Asp Arg Tyr Tyr Arg Glu Pro Phe Val Gln
 340 345 350
 Arg Gln Arg Thr Ser Arg Leu Phe Ser Ile Ser Asn Arg Ile Thr Asp
 355 360 365
 Leu Gly Leu Ser Pro His Leu Thr Ala Leu Met Ala Gly Gly Gln Pro
 370 375 380
 Leu Gly His Ser Ser Ser Thr Gly Asp Thr Gly Phe Ser Cys Ser Gln
 385 390 395 400
 Asp Ser Gly Thr Asp Met Met Leu Leu Glu Asp Tyr Thr Ser Asp Asp
 405 410 415
 Asn Pro Pro Ser His Cys Thr Cys Pro Pro Lys Arg Arg Ser Ser Val
 420 425 430
 Thr Phe Glu Asp Glu Val Glu Gln Ile Lys Glu Ala Ala Lys Asn Phe
 435 440 445
 Asp Ser Ser Cys Glu Ser
 450

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1584 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: Consensus
- (B) CLONE: 1722180

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

GGGACTGACT AAATGTCAGC AAGAGGAGAG TCTCACCTG TTGCTTAGGC AGATGGCGCG      60
ATCTCGGCTC CTGGGTTCAA GCAATTCTCC TGCCTCAGCC TCCTGAATAG CCGGGATTAC      120
AGTCGTCCAG CATGCTCTGC CCACCCACG CCGAGGTGCA CTGACCATGA GCCTCAACTC      180
CTCCCTCAGC TGCAGGAAGG AGCTGAGTAA TCTCACTGAG GGGGAGGGTG GCGAAGGGGG      240
CGTCATCATC ACCCAGTTCA TCGCCATCAT TGTCATCACC ATTTTGTCT GCCTGGGAAA      300
CCTGGTCATC GTGGTCACCT TGTACAAGAA GTCCTACCTC CTCACCTCA GCAACAAGTT      360
CGTCTTCAGC CTGACTCTGT CCAACTTCCT GCTGTCCGTG TTGGTGCTGC CTTTGTGGT      420
GACGAGCTCC ATCCGCAGGG AATGGATCTT TGGTGTAGTG TGGTGCAACT TCTCTGCCCT      480
CCTCTACCTG CTGATCAGCT CTGCCAGCAT GCTAACCTC GGGGTCATTG CCATCGACCG      540
CTACTATGCT GTCCTGTACC CCATGGTGTA CCCCATGAAG ATCACAGGGA ACCGGGCTGT      600
GATGGCACTT GTCTACATCT GGCTTCACTC GCTCATCGGC TGCCTGCCAC CCCTGTTTGG      660
TTGGTCATCC GTGGAGTTTG ACGAGTTCAA ATGGATGTGT GTGGCTGCTT GGCACCGGGA      720
GCCTGGCTAC ACGGCCCTTCT GGCAGATCTG GTGTGCCCTC TTCCCTTTC TGGTCATGCT      780
GGTGTGCTAT GGCTTCATCT TCCGCGTGGC CAGGGTCAAG GCACGCAAGG TGCACTGTGG      840
CACAGTCGTC ATCGTGGAGG AGGATGCTCA GAGGACCGGC GTCCGGAAGA ACTCCAGCAC      900
CTCCACCTCC TCTTCAGGCA GCAGGAGGAA TGCCTTTCAG GGTGTGGTCT ACTCGGCCAA      960
CCAGTGCAAA GCCCTCATCA CCATCCTGGT GGTCTCGGT GCCTTCATGG TCACCTGGGG      1020
CCCCTACATG GTTGTCATCG CCTCTGAGGC CCTCTGGGGG AAAAGCTCCG TCTCCCCGAG      1080
CCTGGAGACT TGGGCCACAT GGCTGTCCTT TGCCAGCGCT GTCTGCCACC CCCTGATCTA      1140
TGGACTCTGG AACAAGACAG TTCGCAAAGA ACTACTGGGC ATGTGCTTTG GGGACCGGTA      1200
TTATCGGGAA CCATTTGTGC AACGACAGAG GACTTCCAGG CTCTTCAGCA TTTCCAACAG      1260
GATCACAGAC CTGGGCCTGT CCCACACCT CACTGCGCTC ATGGCAGGCG GACAGCCCCT      1320
GGGGCACAGC AGCAGCACGG GGGACACTGG CTTCACTGTC TCCCAGGACT CAGGGACAGA      1380
TATGATGCTG CTTGAGGACT ACACGTCTGA TGACAACCT CCCTCTCACT GCACTTGCCC      1440
ACCCAAGAGA AGGAGCTCGG TGACATTTGA GGATGAAGTG GAACAAATCA AAGAAGCTGC      1500
CAAGAACTTC GATTCTTCAT GTGAAAGCTG AAGTACACAA GTCCTTGGAC AGTTACGCAG      1560
CAAGCTTGGC CAAAGCCATT GAGG                                     1584

```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 359 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: GenBank
- (B) CLONE: 163952

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Met Ile Ser Asn Gly Thr Gly Ser Ser Phe Cys Leu Asp Ser Pro Pro
 1           5           10           15
Cys Arg Ile Thr Val Ser Val Val Leu Thr Val Leu Ile Leu Ile Thr
 20           25           30

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Ile Ala Gly Asn Val Val Val Cys Leu Ala Val Gly Leu Asn Arg Arg
   35         40         45
Leu Arg Ser Leu Thr Asn Cys Phe Ile Val Ser Leu Ala Ile Thr Asp
   50         55         60
Leu Leu Leu Gly Leu Leu Val Leu Pro Phe Ser Ala Phe Tyr Gln Leu
   65         70         75         80
Ser Cys Arg Trp Ser Phe Gly Lys Val Phe Cys Asn Ile Tyr Thr Ser
   85         90         95
Leu Asp Val Met Leu Cys Thr Ala Ser Ile Leu Asn Leu Phe Met Ile
  100        105        110
Ser Leu Asp Arg Tyr Cys Ala Val Thr Asp Pro Leu Arg Tyr Pro Val
  115        120        125
Leu Ile Thr Pro Val Arg Val Ala Val Ser Leu Val Leu Ile Trp Val
  130        135        140
Ile Ser Ile Thr Leu Ser Phe Leu Ser Ile His Leu Gly Trp Asn Ser
  145        150        155        160
Arg Asn Glu Thr Ser Ser Phe Asn His Thr Ile Pro Lys Cys Lys Val
  165        170        175
Gln Val Asn Leu Val Tyr Gly Leu Val Asp Gly Leu Val Thr Phe Tyr
  180        185        190
Leu Pro Leu Leu Val Met Cys Ile Thr Tyr Tyr Arg Ile Phe Lys Ile
  195        200        205
Ala Arg Asp Gln Ala Lys Arg Ile His His Met Gly Ser Trp Lys Ala
  210        215        220
Ala Thr Ile Gly Glu His Lys Ala Thr Val Thr Leu Ala Ala Val Met
  225        230        235        240
Gly Ala Phe Ile Ile Cys Trp Phe Pro Tyr Phe Thr Val Phe Val Tyr
  245        250        255
Arg Gly Leu Lys Gly Asp Asp Ala Ile Asn Glu Ala Phe Glu Ala Val
  260        265        270
Val Leu Trp Leu Gly Tyr Ala Asn Ser Ala Leu Asn Pro Ile Leu Tyr
  275        280        285
Ala Thr Leu Asn Arg Asp Phe Arg Thr Ala Tyr Gln Gln Leu Phe Arg
  290        295        300
Cys Arg Pro Ala Ser His Asn Ala Gln Glu Thr Ser Leu Arg Ser Asn
  305        310        315        320
Ser Ser Gln Leu Ala Arg Asn Gln Ser Arg Glu Pro Met Arg Gln Glu
  325        330        335
Glu Lys Pro Leu Lys Leu Gln Val Trp Ser Gly Thr Glu Val Thr Ala
  340        345        350
Pro Arg Gly Ala Thr Asp Arg
  355

```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 359 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: GenBank
- (B) CLONE: 184088

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Ala Pro Asn Gly Thr Ala Ser Ser Phe Cys Leu Asp Ser Thr Ala
 1         5         10         15
Cys Lys Ile Thr Ile Thr Val Val Leu Ala Val Leu Ile Leu Ile Thr
 20        25        30

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Val Ala Gly Asn Val Val Val Cys Leu Ala Val Gly Leu Asn Arg Arg
    35          40          45
Leu Arg Asn Leu Thr Asn Cys Phe Ile Val Ser Leu Ala Ile Thr Asp
    50          55          60
Leu Leu Leu Gly Leu Leu Val Leu Pro Phe Ser Ala Ile Tyr Gln Leu
    65          70          75          80
Ser Cys Lys Trp Ser Phe Gly Lys Val Phe Cys Asn Ile Tyr Thr Ser
    85          90          95
Leu Asp Val Met Leu Cys Thr Ala Ser Ile Leu Asn Leu Phe Met Ile
    100         105         110
Ser Leu Asp Arg Tyr Cys Ala Val Met Asp Pro Leu Arg Tyr Pro Val
    115         120         125
Leu Val Thr Pro Val Arg Val Ala Ile Ser Leu Val Leu Ile Trp Val
    130         135         140
Ile Ser Ile Thr Leu Ser Phe Leu Ser Ile His Leu Gly Trp Asn Ser
    145         150         155         160
Arg Asn Glu Thr Ser Lys Gly Asn His Thr Thr Ser Lys Cys Lys Val
    165         170         175
Gln Val Asn Glu Val Tyr Gly Leu Val Asp Gly Leu Val Thr Phe Tyr
    180         185         190
Leu Pro Leu Leu Ile Met Cys Ile Thr Tyr Tyr Arg Ile Phe Lys Val
    195         200         205
Ala Arg Asp Gln Ala Lys Arg Ile Asn His Ile Ser Ser Trp Lys Ala
    210         215         220
Ala Thr Ile Arg Glu His Lys Ala Thr Val Thr Leu Ala Ala Val Met
    225         230         235         240
Gly Ala Phe Ile Ile Cys Trp Phe Pro Tyr Phe Thr Ala Phe Val Tyr
    245         250         255
Arg Gly Leu Arg Gly Asp Asp Ala Ile Asn Glu Val Leu Glu Ala Ile
    260         265         270
Val Leu Trp Leu Gly Tyr Ala Asn Ser Ala Leu Asn Pro Ile Leu Tyr
    275         280         285
Ala Ala Leu Asn Arg Asp Phe Arg Thr Gly Tyr Gln Gln Leu Phe Cys
    290         295         300
Cys Arg Leu Ala Asn Arg Asn Ser His Lys Thr Ser Leu Arg Ser Asn
    305         310         315         320
Ala Ser Gln Leu Ser Arg Thr Gln Ser Arg Glu Pro Arg Gln Gln Glu
    325         330         335
Glu Lys Pro Leu Lys Leu Gln Val Trp Ser Gly Thr Glu Val Thr Ala
    340         345         350
Pro Gln Gly Ala Thr Asp Arg
    355

```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 359 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: GenBank
- (B) CLONE: 791239

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

Met Ala Phe Asn Gly Thr Val Pro Ser Phe Cys Met Asp Phe Thr Val
  1          5          10          15
Tyr Lys Val Thr Ile Ser Val Ile Leu Ile Ile Leu Ile Leu Val Thr
    20          25          30

```

```

Val Ala Gly Asn Val Val Val Cys Leu Ala Val Gly Leu Asn Arg Arg
    35          40          45
Leu Arg Ser Leu Thr Asn Cys Phe Ile Val Ser Leu Ala Val Thr Asp
    50          55          60
Leu Leu Leu Gly Leu Leu Val Leu Pro Phe Ser Ala Ile Tyr Gln Leu
    65          70          75          80
Ser Cys Lys Trp Ser Phe Ser Lys Val Phe Cys Asn Ile Tyr Thr Ser
    85          90          95
Leu Asp Val Met Leu Cys Thr Ala Ser Ile Leu Asn Leu Phe Met Ile
    100          105          110
Ser Leu Asp Arg Tyr Cys Ala Val Thr Asp Pro Leu Arg Tyr Pro Val
    115          120          125
Leu Ile Thr Pro Ala Arg Val Ala Ile Ser Leu Val Phe Ile Trp Val
    130          135          140
Ile Ser Ile Thr Leu Ser Phe Leu Ser Ile His Leu Gly Trp Asn Ser
    145          150          155          160
Arg Asn Glu Thr Ser Lys Asp Asn Asp Thr Ile Val Lys Cys Lys Val
    165          170          175
Gln Val Asn Glu Val Tyr Gly Leu Val Asp Gly Leu Val Thr Phe Tyr
    180          185          190
Leu Pro Leu Leu Ile Met Cys Ile Thr Tyr Phe Arg Ile Phe Lys Ile
    195          200          205
Ala Arg Glu Gln Ala Arg Arg Ile Asn His Ile Gly Ser Trp Lys Ala
    210          215          220
Ala Thr Ile Arg Glu His Lys Ala Thr Val Thr Leu Ala Ala Val Met
    225          230          235          240
Gly Ala Phe Ile Ile Cys Trp Phe Pro Tyr Phe Thr Val Phe Val Tyr
    245          250          255
Arg Gly Leu Lys Gly Asp Asp Ala Val Asn Glu Val Phe Glu Asp Val
    260          265          270
Val Leu Trp Leu Gly Tyr Ala Asn Ser Ala Leu Asn Pro Ile Leu Tyr
    275          280          285
Ala Ala Leu Asn Arg Asp Phe Arg Thr Ala Tyr His Gln Leu Phe Cys
    290          295          300
Cys Arg Leu Ala Ser His Asn Ser His Glu Thr Ser Leu Arg Leu Asn
    305          310          315          320
Asn Ser Gln Leu Asn Arg Ser Gln Cys Gln Glu Pro Arg Trp Gln Glu
    325          330          335
Asp Lys Pro Leu Asn Leu Gln Val Trp Ser Gly Thr Glu Val Thr Ala
    340          345          350
Pro Gln Gly Ala Thr Asn Arg
    355

```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 358 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: GenBank
- (B) CLONE: 236184

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Met Glu Pro Asn Gly Thr Val His Ser Cys Cys Leu Asp Ser Met Ala
  1          5          10          15
Leu Lys Val Thr Ile Ser Val Val Leu Thr Thr Leu Ile Leu Ile Thr
    20          25          30

```

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Ile Ala Gly Asn Val Val Val Cys Leu Ala Val Ser Leu Asn Arg Arg
    35          40          45
Leu Arg Ser Leu Thr Asn Cys Phe Ile Val Ser Leu Ala Ala Thr Asp
    50          55          60
Leu Leu Leu Gly Leu Leu Val Leu Pro Phe Ser Ala Ile Tyr Gln Leu
    65          70          75          80
Ser Phe Thr Trp Ser Phe Gly His Val Phe Cys Asn Ile Tyr Thr Ser
    85          90          95
Leu Asp Val Met Leu Cys Thr Ala Ser Ile Leu Asn Leu Phe Met Ile
    100         105         110
Ser Leu Asp Arg Tyr Cys Ala Val Thr Asp Pro Leu Arg Tyr Pro Val
    115         120         125
Leu Val Thr Pro Val Arg Val Ala Ile Ser Leu Val Phe Ile Trp Val
    130         135         140
Ile Ser Ile Thr Leu Ser Phe Leu Ser Ile His Leu Gly Trp Asn Ser
    145         150         155         160
Arg Asn Gly Thr Arg Gly Gly Asn Asp Thr Phe Lys Cys Lys Val Gln
    165         170         175
Val Asn Glu Val Tyr Gly Leu Val Asp Gly Leu Val Thr Phe Tyr Leu
    180         185         190
Pro Leu Leu Ile Met Cys Val Thr Tyr Tyr Arg Ile Phe Lys Ile Ala
    195         200         205
Arg Glu Gln Ala Lys Arg Ile Asn His Ile Ser Ser Trp Lys Ala Ala
    210         215         220
Thr Ile Arg Glu His Lys Ala Thr Val Thr Leu Ala Ala Val Met Gly
    225         230         235         240
Ala Phe Ile Ile Cys Trp Phe Pro Tyr Phe Thr Ala Phe Val Tyr Arg
    245         250         255
Gly Leu Arg Gly Asp Asp Ala Ile Asn Glu Ala Val Glu Gly Ile Val
    260         265         270
Leu Trp Leu Gly Tyr Ala Asn Ser Ala Leu Asn Pro Ile Leu Tyr Ala
    275         280         285
Ala Leu Asn Arg Asp Phe Arg Thr Ala Tyr Gln Gln Leu Phe His Cys
    290         295         300
Lys Phe Ala Ser His Asn Ser His Lys Thr Ser Leu Arg Leu Asn Asn
    305         310         315         320
Ser Leu Leu Pro Arg Ser Gln Ser Arg Glu Gly Arg Trp Gln Glu Glu
    325         330         335
Lys Pro Leu Lys Leu Gln Val Trp Ser Gly Thr Glu Leu Thr His Pro
    340         345         350
Gln Gly Asn Pro Ile Arg
    355

```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 429 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: GenBank
- (B) CLONE: 927211

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

Met Val Phe Leu Ser Gly Asn Ala Ser Asp Ser Ser Asn Cys Thr Gln
  1          5          10          15
Pro Pro Ala Pro Val Asn Ile Ser Lys Ala Ile Leu Leu Gly Val Ile
    20          25          30

```

```

Leu Gly Gly Leu Ile Leu Phe Gly Val Leu Gly Asn Ile Leu Val Ile
   35         40         45
Leu Ser Val Ala Cys His Arg His Leu His Ser Val Thr His Tyr Tyr
   50         55         60
Ile Val Asn Leu Ala Val Ala Asp Leu Leu Leu Thr Ser Thr Val Leu
   65         70         75         80
Pro Phe Ser Ala Ile Phe Glu Val Leu Gly Tyr Trp Ala Phe Gly Arg
   85         90         95
Val Phe Cys Asn Ile Trp Ala Ala Val Asp Val Leu Cys Cys Thr Ala
   100        105        110
Ser Ile Met Gly Leu Cys Ile Ile Ser Ile Asp Arg Tyr Ile Gly Val
   115        120        125
Ser Tyr Pro Leu Arg Tyr Pro Thr Ile Val Thr Gln Arg Arg Gly Leu
   130        135        140
Met Ala Leu Leu Cys Val Trp Ala Leu Ser Leu Val Ile Ser Ile Gly
   145        150        155        160
Pro Leu Phe Gly Trp Arg Gln Pro Ala Pro Glu Asp Glu Thr Ile Cys
   165        170        175
Gln Ile Asn Glu Glu Pro Gly Tyr Val Leu Phe Ser Ala Leu Gly Ser
   180        185        190
Phe Tyr Leu Pro Leu Ala Ile Ile Leu Val Met Tyr Cys Arg Val Tyr
   195        200        205
Val Val Ala Lys Arg Glu Ser Arg Gly Leu Lys Ser Gly Leu Lys Thr
   210        215        220
Asp Lys Ser Asp Ser Glu Gln Val Thr Leu Arg Ile His Arg Lys Asn
   225        230        235        240
Ala Pro Ala Gly Gly Ser Gly Met Ala Ser Ala Lys Thr Lys Thr His
   245        250        255
Phe Ser Val Arg Leu Leu Lys Phe Ser Arg Glu Lys Lys Ala Ala Lys
   260        265        270
Thr Leu Gly Ile Val Val Gly Cys Phe Val Leu Cys Trp Leu Pro Phe
   275        280        285
Phe Leu Val Met Pro Ile Gly Ser Phe Phe Pro Asp Phe Lys Pro Ser
   290        295        300
Glu Thr Val Phe Lys Ile Val Phe Trp Leu Gly Tyr Leu Asn Ser Cys
   305        310        315        320
Ile Asn Pro Ile Ile Tyr Pro Cys Ser Ser Gln Glu Phe Lys Lys Ala
   325        330        335
Phe Gln Asn Val Leu Arg Ile Gln Cys Leu Arg Arg Lys Gln Ser Ser
   340        345        350
Lys His Ala Leu Gly Tyr Thr Leu His Pro Pro Ser Gln Ala Val Glu
   355        360        365
Gly Gln His Lys Asp Met Val Arg Ile Pro Val Gly Ser Arg Glu Thr
   370        375        380
Phe Tyr Arg Ile Ser Lys Thr Asp Gly Val Cys Glu Trp Lys Phe Phe
   385        390        395        400
Ser Ser Met Pro Arg Gly Ser Ala Arg Ile Thr Val Ser Lys Asp Gln
   405        410        415
Ser Ser Cys Thr Thr Ala Arg Gly His Thr Pro Met Thr
   420        425

```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 481 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: GenBank

(B) CLONE: 475198

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```

Met Ala Leu Ser Tyr Arg Val Ser Glu Leu Gln Ser Thr Ile Pro Glu
 1      5      10      15
His Ile Leu Gln Ser Thr Phe Val His Val Ile Ser Ser Asn Trp Ser
      20      25      30
Gly Leu Gln Thr Glu Ser Ile Pro Glu Glu Met Lys Gln Ile Val Glu
      35      40      45
Glu Gln Gly Asn Lys Leu His Trp Ala Ala Leu Leu Ile Leu Met Val
      50      55      60
Ile Ile Pro Thr Ile Gly Gly Asn Thr Leu Val Ile Leu Ala Val Ser
      65      70      75      80
Leu Glu Lys Lys Leu Gln Tyr Ala Thr Asn Tyr Phe Leu Met Ser Leu
      85      90      95
Ala Val Ala Asp Leu Leu Val Gly Leu Phe Val Met Pro Ile Ala Leu
      100      105      110
Leu Thr Ile Met Phe Glu Ala Met Trp Pro Leu Pro Leu Val Leu Cys
      115      120      125
Pro Ala Trp Leu Phe Leu Asp Val Leu Phe Ser Thr Ala Ser Ile Met
      130      135      140
His Leu Cys Ala Ile Ser Val Asp Arg Tyr Ile Ala Ile Lys Lys Pro
      145      150      155      160
Ile Gln Ala Asn Gln Tyr Asn Ser Arg Ala Thr Ala Phe Ile Lys Ile
      165      170      175
Thr Val Val Trp Leu Ile Ser Ile Gly Ile Ala Ile Pro Val Pro Ile
      180      185      190
Lys Gly Ile Glu Thr Asp Val Asp Asn Pro Asn Asn Ile Thr Cys Val
      195      200      205
Leu Thr Lys Glu Arg Phe Gly Asp Phe Met Leu Phe Gly Ser Leu Ala
      210      215      220
Ala Phe Phe Thr Pro Leu Ala Ile Met Ile Val Thr Tyr Phe Leu Thr
      225      230      235      240
Ile His Ala Leu Gln Lys Lys Ala Tyr Leu Val Lys Asn Lys Pro Pro
      245      250      255
Gln Arg Leu Thr Trp Leu Thr Val Ser Thr Val Phe Gln Arg Asp Glu
      260      265      270
Thr Pro Cys Ser Ser Pro Glu Lys Val Ala Met Leu Asp Gly Ser Arg
      275      280      285
Lys Asp Lys Ala Leu Pro Asn Ser Gly Asp Glu Thr Leu Met Arg Arg
      290      295      300
Thr Ser Thr Ile Gly Lys Lys Ser Val Gln Thr Ile Ser Asn Glu Gln
      305      310      315      320
Arg Ala Ser Lys Val Leu Gly Ile Val Phe Phe Leu Phe Leu Leu Met
      325      330      335
Trp Cys Pro Phe Phe Ile Thr Asn Ile Thr Leu Val Leu Cys Asp Ser
      340      345      350
Cys Asn Gln Thr Thr Leu Gln Met Leu Leu Glu Ile Phe Val Trp Ile
      355      360      365
Gly Tyr Val Ser Ser Gly Val Asn Pro Leu Val Tyr Thr Leu Phe Asn
      370      375      380
Lys Thr Phe Arg Asp Ala Phe Gly Arg Tyr Ile Thr Cys Asn Tyr Arg
      385      390      395      400
Ala Thr Lys Ser Val Lys Thr Leu Arg Lys Arg Ser Ser Lys Ile Tyr
      405      410      415
Phe Arg Asn Pro Met Ala Glu Asn Ser Lys Phe Phe Lys Lys His Gly
      420      425      430
Ile Arg Asn Gly Ile Asn Pro Ala Met Tyr Gln Ser Pro Met Arg Leu
      435      440      445

```

Arg Ser Ser Thr Ile Gln Ser Ser Ser Ile Ile Leu Leu Asp Thr Leu
450 455 460
Leu Leu Thr Glu Asn Glu Gly Asp Lys Thr Glu Glu Gln Val Ser Tyr
465 470 475 480
Val

What is claimed is:

1. A substantially purified histamine H2 receptor (H2RH) comprising the amino acid sequence of SEQ ID NO:1 or fragments thereof.
2. An isolated and purified polynucleotide sequence encoding the histamine H2 receptor of claim 1.
3. A polynucleotide sequence which hybridizes under stringent conditions to the polynucleotide sequence of claim 2.
4. A hybridization probe comprising the polynucleotide sequence of claim 2.
5. An isolated and purified polynucleotide sequence comprising SEQ ID NO:2 or variants thereof.
6. A polynucleotide sequence which is complementary to SEQ ID NO:2 or variants thereof.
7. A hybridization probe comprising the polynucleotide sequence of claim 6.
8. An expression vector containing the polynucleotide sequence of claim 2.
9. A host cell containing the vector of claim 8.
10. A method for producing a polypeptide comprising the amino acid sequence of SEQ ID NO:1 the method comprising the steps of:
 - a) culturing the host cell of claim 9 under conditions suitable for the expression of the polypeptide; and
 - b) recovering the polypeptide from the host cell culture.
11. A purified antibody which binds specifically to the polypeptide of claim 1.
12. A method for detecting and quantifying the polypeptide of SEQ ID NO:1 in a biological sample, the method comprising:
 - combining the biological sample with the purified antibody of claim 11 under conditions suitable for binding,
 - detecting the bound polypeptide:antibody,
 - quantifying the amount of polypeptide:antibody, thereby establishing the presence and amount of the polypeptide in the sample.
13. A purified agonist which specifically binds to and modulates the activity of the polypeptide of claim 1.

14. A purified antagonist which specifically binds to and modulates the activity of the polypeptide of claim 1.

15. A pharmaceutical composition comprising the antagonist of claim 14 and a pharmaceutically acceptable excipient.

5 16. A method for treating inflammatory disease comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 15.

17. A method for treating gastric conditions comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 15.

10 18. A method for treating nervous conditions comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 15.

19. A method for detection of polynucleotides encoding histamine H2 receptor of claim 1 in a biological sample comprising the steps of:

a) hybridizing a polynucleotide consisting of SEQ ID NO:2 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and

15 b) detecting said hybridization complex, wherein the presence of said complex correlates with the presence of a polynucleotide encoding histamine H2 receptor in said biological sample.

20. The method of claim 19 wherein before hybridization, the nucleic acid material of the biological sample is amplified by the polymerase chain reaction.

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5'	9	18	27	36	45	54
NNG GGA CTG ACT AAA TGT CAG CAA GAG GAG AGT CTC ACC CTG TTG CTT AGG CAG						
	63	72	81	90	99	108
ATG GCG CGA TCT CGG CTC CTG GGT TCA AGC AAT TCT CCT GCC TCA GCC TCC TGA						
	117	126	135	144	153	162
ATA GCC GGG ATT ACA GTC GTC CAG CAT GCT CTG CCC ACC CCA CGC CGA GGT GCA						
	171	180	189	198	207	216
CTG ACC ATG AGC CTC AAC TCC TCC CTC AGC TGC AGG AAG GAG CTG AGT AAT CTC						
M S L N S S L S C R K E L S N L						
	225	234	243	252	261	270
ACT GAG GGG GAG GGT GGC GAA GGG GGC GTC ATC ATC ACC CAG TTC ATC GCC ATC						
T E G E G G E G G G V I I T Q F I A I						
	279	288	297	306	315	324
ATT GTC ATC ACC ATT TTT GTC TGC TGC GGA AAC CTG GTC ATC GTG GTC ACC TTG						
I V I T I F V C L G N L V I V V T L						
	333	342	351	360	369	378
TAC AAG AAG TCC TAC CTC ACC CTC AGC AAC AAG TTC GTC TTC AGC CTG ACT						
Y K K S Y L L T L S N K F V F S L T						
	387	396	405	414	423	432
CTG TCC AAC TTC CTG CTG TCC GTG TTG GTG CTG CCT TTT GTG GTG ACG AGC TCC						
L S N F L L S V L V L P F V V T S S						

FIGURE 1A

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441 450 459 468 477 486
ATC CGC AGG GAA TGG ATC TTT GGT GTA GTG TGG TGC AAC TTC TCT GCC CTC CTC
I R R E W I F G V V W C N F S A L L

495 504 513 522 531 540
TAC CTG CTG ATC AGC TCT GCC AGC ATG CTA ACC CTC GGG GTC ATT GCC ATC GAC
Y L L I S S A S M L T L G V I A I D

549 558 567 576 585 594
CGC TAC TAT GCT GTC CTG TAC CCC ATG GTG TAC CCC ATG AAG ATC ACA GGG AAC
R Y Y A V L Y P M V Y P M K I T G N

603 612 621 630 639 648
CGG GCT GTG ATG GCA CTT GTC TAC ATC TGG CTT CAC TCG CTC ATC GGC TGC CTG
R A V M A L V Y I W L H S L I G C L

657 666 675 684 693 702
CCA CCC CTG TTT GGT TGG TCA TCC GTG GAG TTT GAC GAG TTC AAA TGG ATG TGT
P P L F G W S S V E F D E F K W M C

711 720 729 738 747 756
GTG GCT GCT TGG CAC CGG GAG CCT GGC TAC ACG GCC TTC TGG CAG ATC TGG TGT
V A A W H R E P G Y T A F W Q I W C

765 774 783 792 801 810
GCC CTC TTC CCC TTT CTG GTC ATG CTG GTG TGC TAT GGC TTC ATC TTC CGC GTG
A L F P P F L V M L V C Y G F I F R V

FIGURE 1B

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819	828	837	846	855	864
GCC AGG GTC AAG GCA CGC AAG GTG CAC TGT GGC ACA GTC GTC ATC GTG GAG GAG					
A R V K A R K V H C G T V I V E E					
873	882	891	900	909	918
GAT GCT CAG AGG ACC GGC GTC CGG AAG AAC TCC AGC ACC TCC ACC TCC TCT TCA					
D A Q R R T G V R K N S S T S S S					
927	936	945	954	963	972
GGC AGC AGG AGG AAT GCC TTT CAG GGT GTG GTC TAC TCG GCC AAC CAG TGC AAA					
G S R R R N A F Q G V Y S A N Q C K					
981	990	999	1008	1017	1026
GCC CTC ATC ACC ATC CTG GTG GTC CTC GGT GCC TTC ATG GTC ACC TGG GGC CCC					
A L I T I L V V L G A F M V T W G P					
1035	1044	1053	1062	1071	1080
TAC ATG GTT GTC ATC GCC TCT GAG GCC CTC TGG GGG AAA AGC TCC GTC TCC CCG					
Y M V V I A S E A L W G K S V S P					
1089	1098	1107	1116	1125	1134
AGC CTG GAG ACT TGG GCC ACA TGG CTG TCC TTT GCC AGC GCT GTC TGC CAC CCC					
S L E T W A T W L S F A S A V C H P					
1143	1152	1161	1170	1179	1188
CTG ATC TAT GGA CTC TGG AAC AAG ACA GTT CGC AAA GAA CTA CTG GGC ATG TGC					
L I Y G L W N K T V R K E L L G M C					

FIGURE 1C

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1197 1206 1215 1224 1233 1242
TTT GGG GAC CGG TAT TAT CGG GAA CCA TTT GTG CAA CGA CAG AGG ACT TCC AGG
F G D R Y Y R E P F V Q R Q R T S R

1251 1260 1269 1278 1287 1296
CTC TTC AGC ATT TCC AAC AGG ATC ACA GAC CTG GGC CTG TCC CCA CAC CTC ACT
L F S I S N R I T D L G L S P H L T

1305 1314 1323 1332 1341 1350
GCG CTC ATG GCA GGC GGA CAG CCC CTG GGG CAC AGC AGC AGC GGG GAC ACT
A L M A G G Q P L G H S S S T G D T

1359 1368 1377 1386 1395 1404
GGC TTC AGC TGC TCC CAG GAC TCA GGG ACA GAT ATG CTG CTT GAG GAC TAC
G F S C S Q D S G T D M M L L E D Y

1413 1422 1431 1440 1449 1458
ACG TCT GAT GAC AAC CCT CCC TCT CAC TGC ACT TGC CCA CCC AAG AGA AGG AGC
T S D D N P P S H C T C P P K R R S

1467 1476 1485 1494 1503 1512
TCG GTG ACA TTT GAG GAT GAA GTG GAA CAA ATC AAA GAA GCT GCC AAG AAC TTC
S V T F E D E V E Q I K E A A K N F

1521 1530 1539 1548 1557 1566
GAT TCT TCA TGT GAA AGC TGA AGT ACA CAA GTC CTT GGA CAG TTA CGC AGC AAG
D S S C C E S

1575 1584
CTT GGC CAA AGC CAT TGA GG 3'

FIGURE 1D

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[illegible]

54	K	K	S	Y	L	L	T	L	S	N	K	F	V	F	S	L	T	L	S	N	F	L	L	S	V	L	V	L	P	F	-	V	V	T	S	S	I	R	R	E	1722180		
45	L	N	R	R	L	L	R	S	L	T	N	C	F	I	V	S	L	A	I	T	D	L	L	L	G	L	L	V	L	P	F	S	A	F	-	Y	Q	L	S	C	R	GI 163952	
42	L	N	R	R	L	L	R	N	L	T	N	C	F	I	V	S	L	A	I	T	D	L	L	L	G	L	L	V	L	P	F	S	A	I	-	Y	Q	L	S	C	K	GI 1355759	
45	L	N	R	R	L	L	R	N	L	T	N	C	F	I	V	S	L	A	I	T	D	L	L	L	G	L	L	V	L	P	F	S	A	I	-	Y	Q	L	S	C	K	GI 184088	
45	L	N	R	R	L	L	R	S	L	T	N	C	F	I	V	S	L	A	V	T	D	L	L	L	G	L	L	V	L	P	F	S	A	I	-	Y	Q	L	S	C	K	GI 791239	
45	L	N	R	R	L	L	R	S	L	T	N	C	F	I	V	S	L	A	A	T	D	L	L	L	G	L	L	V	L	P	F	S	A	I	-	Y	Q	L	S	F	T	GI 236184	
53	C	H	R	H	L	L	H	S	V	T	H	Y	Y	I	V	N	L	A	V	A	D	L	L	L	T	S	T	V	L	P	F	S	A	I	-	F	E	V	L	G	Y	GI 927211	
81	L	E	K	K	L	L	Q	Y	A	T	N	Y	F	L	M	S	L	A	V	A	D	L	L	L	V	G	L	F	V	M	P	I	A	L	L	T	I	M	F	E	A	M	GI 475198

FIGURE 2A

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93	W	I	F	G	V	V	C	N	F	S	A	L	L	Y	L	L	I	S	S	A	S	M	L	T	L	G	V	I	A	I	D	R	Y	Y	A	V	L	Y	P	1722180			
84	W	S	F	G	K	V	F	C	N	I	Y	T	S	L	D	V	M	L	C	T	A	S	I	L	N	L	F	M	I	S	L	D	R	Y	C	A	V	T	D	P	GI 163952		
81	W	S	F	G	K	V	F	C	N	I	Y	T	S	L	D	V	M	L	C	T	A	S	I	L	N	L	F	M	I	S	L	D	R	Y	C	A	V	M	D	P	GI 1355759		
84	W	S	F	G	K	V	F	C	N	I	Y	T	S	L	D	V	M	L	C	T	A	S	I	L	N	L	F	M	I	S	L	D	R	Y	C	A	V	M	D	P	GI 184088		
84	W	S	F	G	K	V	F	C	N	I	Y	T	S	L	D	V	M	L	C	T	A	S	I	L	N	L	F	M	I	S	L	D	R	Y	C	A	V	T	D	P	GI 791239		
84	W	S	F	G	H	V	F	C	N	I	Y	T	S	L	D	V	M	L	C	T	A	S	I	L	N	L	F	M	I	S	L	D	R	Y	C	A	V	T	D	P	GI 236184		
92	W	A	F	G	R	V	F	C	N	I	W	A	V	D	V	L	C	C	T	A	S	I	M	G	L	C	I	I	S	I	D	R	Y	I	G	V	S	Y	P	GI 927211			
121	W	P	L	P	L	V	L	C	P	A	W	L	F	L	D	V	L	F	S	T	A	S	I	M	H	L	C	A	I	S	V	D	R	Y	I	A	I	K	K	P	GI 475198		
133	M	V	Y	P	M	K	I	T	G	N	R	A	V	M	A	L	V	Y	I	W	L	H	S	L	-	I	G	C	L	P	P	L	F	G	W	S	S	V	E	-	1722180		
124	L	R	Y	P	V	L	I	T	P	V	R	V	A	V	S	L	V	L	I	W	V	I	S	I	T	L	S	F	L	S	I	H	L	G	W	N	S	R	N	E	GI 163952		
121	L	R	Y	P	V	L	V	T	P	A	R	V	A	I	S	L	V	L	I	W	V	I	S	I	T	L	S	F	L	S	I	H	L	G	W	N	S	R	N	E	GI 1355759		
124	L	R	Y	P	V	L	V	T	P	A	R	V	A	I	S	L	V	L	I	W	V	I	S	I	T	L	S	F	L	S	I	H	L	G	W	N	S	R	N	E	GI 184088		
124	L	R	Y	P	V	L	I	T	P	A	R	V	A	I	S	L	V	F	I	W	V	I	S	I	T	L	S	F	L	S	I	H	L	G	W	N	S	R	N	E	GI 791239		
124	L	R	Y	P	V	L	V	T	P	V	R	V	A	I	S	L	V	F	I	W	V	I	S	I	T	L	S	F	L	S	I	H	L	G	W	N	S	R	N	E	GI 236184		
132	L	R	Y	P	T	I	V	T	Q	R	R	G	L	M	A	L	L	C	V	W	A	L	S	L	V	I	S	I	G	P	L	-	F	G	W	-	-	R	Q	GI 927211			
161	I	Q	A	N	Q	Y	N	S	R	A	T	A	F	I	K	I	T	V	V	W	L	I	S	I	G	I	A	I	-	P	V	P	I	-	-	K	G	I	E	GI 475198			
171	-	-	-	F	D	E	F	K	W	M	C	V	A	A	W	H	R	E	P	G	Y	T	A	F	W	Q	I	W	C	A	L	F	P	F	L	V	M	L	V	C	1722180		
164	T	S	S	F	N	H	T	I	P	K	C	K	V	Q	V	N	-	-	L	V	Y	G	L	V	D	G	L	V	T	F	Y	L	P	L	P	L	L	V	M	C	I	T	GI 163952
161	T	S	K	G	N	H	T	T	S	K	C	N	V	Q	V	N	-	-	E	V	Y	G	L	V	D	G	L	V	T	F	Y	L	P	L	P	L	L	I	M	C	I	T	GI 1355759
164	T	S	K	G	N	H	T	T	S	K	C	K	V	Q	V	N	-	-	E	V	Y	G	L	V	D	G	L	V	T	F	Y	L	P	L	P	L	L	I	M	C	I	T	GI 184088
164	T	S	K	D	N	D	T	I	V	K	C	K	V	Q	V	N	-	-	E	V	Y	G	L	V	D	G	L	V	T	F	Y	L	P	L	P	L	L	I	M	C	I	T	GI 791239
164	T	R	G	G	N	D	T	F	-	K	C	K	V	Q	V	N	-	-	E	V	Y	G	L	V	D	G	L	V	T	F	Y	L	P	L	P	L	L	I	M	C	I	T	GI 236184
168	P	A	P	E	D	E	T	I	-	-	C	Q	I	N	E	E	-	-	P	G	Y	V	L	F	S	A	L	G	S	F	Y	L	P	L	P	L	A	I	L	V	M	GI 927211	
197	T	D	V	D	N	P	N	I	T	-	C	V	L	T	K	E	R	F	G	D	F	M	L	F	G	S	L	A	A	F	F	T	P	L	P	L	A	I	M	I	V	T	GI 475198

FIGURE 2B

208	YGF	-	-	-	-	I	F	R	V	A	R	V	K	A	R	K	V	H	-	-	-	-	-	-	-	C	G	1722180															
202	Y Y	-	-	-	-	R	I	F	K	I	A	R	D	Q	A	K	-	-	-	-	-	-	-	-	-	-	-	GI 163952															
199	Y Y	-	-	-	-	R	I	F	R	V	A	R	D	Q	A	K	-	-	-	-	-	-	-	-	-	-	-	GI 1355759															
202	Y Y	-	-	-	-	R	I	F	K	V	A	R	D	Q	A	K	-	-	-	-	-	-	-	-	-	-	-	GI 184088															
202	Y F	-	-	-	-	R	I	F	K	I	A	R	E	Q	A	R	-	-	-	-	-	-	-	-	-	-	-	GI 791239															
201	Y Y	-	-	-	-	R	I	F	K	I	A	R	E	Q	A	K	-	-	-	-	-	-	-	-	-	-	-	GI 236184															
204	Y C	-	-	-	-	R	V	Y	V	V	A	K	R	E	S	R	G	L	K	T	D	K	-	-	-	S	-	GI 927211															
237	Y F L T I H A L Q K K A Y L V	-	-	-	-	V	K	N	K	P	P	Q	R	L	T	W	L	T	V	S	T	V	F	Q	R	D	E	T	P	C	S	GI 475198											
226	T V - -	-	-	-	-	V	I	V	E	E	D	A	O	R	T	G	V	R	K	N	S	S	T	S	T	S	S	G	S	R	R	N	A	F	Q	G	V	V	Y	S	1722180		
215	-	-	-	-	-	R	I	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	M	G	S	W	K	A	-	-	A	T	GI 163952			
212	-	-	-	-	-	R	I	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	T	GI 1355759			
215	-	-	-	-	-	R	I	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	T	GI 184088			
215	-	-	-	-	-	R	I	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	T	GI 791239			
214	-	-	-	-	-	R	I	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	T	GI 236184			
228	D S E Q V	-	-	-	-	T	-	L	R	I	H	R	K	N	A	P	A	G	S	G	M	A	S	A	K	T	K	T	H	F	S	V	R	L	L	-	-	-	K	F	GI 927211		
277	S P E K V	-	-	-	-	A	M	L	D	G	S	R	K	D	K	A	L	P	N	S	G	D	E	T	L	M	R	R	T	S	T	I	G	K	K	S	V	Q	T	I	GI 475198		
264	A N O C K A L I T I L V V L G A F M V T W G P Y M V V I A S E A L W G	-	-	-	-	T	V	A	M	L	D	G	S	R	K	D	K	A	L	P	N	S	G	D	E	T	L	M	R	R	T	S	T	I	G	K	K	S	V	Q	T	I	GI 475198
227	I G E H K A T V T L A A V M G A F I I C W F P Y F P Y F T V F V Y R G L K G	-	-	-	-	T	V	A	M	L	D	G	S	R	K	D	K	A	L	P	N	S	G	D	E	T	L	M	R	R	T	S	T	I	G	K	K	S	V	Q	T	I	GI 475198
224	I R E H R A T V T L A A V M G A F I I C W F P Y F T V F V Y R G L R G	-	-	-	-	T	V	A	M	L	D	G	S	R	K	D	K	A	L	P	N	S	G	D	E	T	L	M	R	R	T	S	T	I	G	K	K	S	V	Q	T	I	GI 475198
227	I R E H K A T V T L A A V M G A F I I C W F P Y F T V F V Y R G L R G	-	-	-	-	T	V	A	M	L	D	G	S	R	K	D	K	A	L	P	N	S																					

FIGURE 2C

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302	V	S	P	S	L	E	T	W	A	T	W	L	S	F	A	S	A	V	C	H	P	L	I	Y	G	L	W	N	K	T	V	R	K	E	L	L	G	M	C	F	1722180								
265	I	N	E	A	F	E	A	V	V	L	W	L	G	Y	A	N	S	A	L	N	P	I	L	Y	A	T	L	N	R	D	F	R	T	A	-	-	-	-	Y	GI 163952									
262	I	N	E	M	L	E	A	I	V	L	W	L	G	Y	A	N	S	A	L	N	P	I	L	Y	A	A	L	N	R	D	F	R	T	G	-	-	-	-	Y	GI 1355759									
265	I	N	E	V	L	E	A	I	V	L	W	L	G	Y	A	N	S	A	L	N	P	I	L	Y	A	A	L	N	R	D	F	R	T	G	-	-	-	-	Y	GI 184088									
265	V	N	E	V	F	E	D	V	V	L	W	L	G	Y	A	N	S	A	L	N	P	I	L	Y	A	A	L	N	R	D	F	R	T	A	-	-	-	-	Y	GI 791239									
264	I	N	E	A	V	E	G	I	V	L	W	L	G	Y	A	N	S	A	L	N	P	I	L	Y	A	A	L	N	R	D	F	R	T	A	-	-	-	-	Y	GI 236184									
303	P	S	E	T	V	F	K	I	V	F	W	L	G	Y	L	N	S	C	I	N	P	I	I	Y	P	C	S	S	Q	E	F	K	K	A	-	-	-	-	F	GI 927211									
357	T	L	Q	M	L	L	E	I	F	V	W	I	G	Y	V	S	S	G	V	N	P	L	V	Y	T	L	F	N	K	T	F	R	D	A	-	-	-	-	F	GI 475198									
342	G	D	R	Y	Y	R	E	P	F	V	Q	R	Q	R	T	S	R	L	F	S	I	S	N	R	I	T	D	L	-	G	L	S	P	H	L	T	A	L	M	A	1722180								
300	Q	Q	L	F	R	-	-	C	R	P	A	S	H	N	A	Q	E	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	L	R	S	GI 163952					
297	Q	Q	L	F	C	-	-	C	R	L	A	N	R	N	S	H	K	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	L	R	S	GI 1355759			
300	Q	Q	L	F	C	-	-	C	R	L	A	N	R	N	S	H	K	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	L	R	S	GI 184088			
300	H	Q	L	F	C	-	-	C	R	L	A	S	H	N	S	H	E	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	L	R	L	GI 791239		
299	Q	Q	L	F	H	-	-	C	K	F	A	S	H	N	S	H	K	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	L	R	L	GI 236184		
338	Q	N	V	L	R	I	Q	C	L	R	R	K	Q	S	S	K	H	-	-	A	L	G	-	-	-	-	-	-	Y	T	L	H	P	P	S	Q	A	V	E	G	GI 927211								
392	G	R	Y	-	-	I	T	C	N	Y	R	A	T	K	S	V	K	-	-	T	L	R	K	R	S	S	K	I	Y	F	R	N	P	M	A	E	N	S	K	F	GI 475198								
381	G	G	Q	P	L	G	H	S	S	S	T	G	D	T	G	F	S	C	S	Q	D	S	G	T	D	M	M	L	L	E	D	Y	T	S	D	D	N	P	P	S	1722180								
320	N	S	S	Q	L	A	R	N	Q	-	-	S	R	E	P	M	R	-	Q	E	E	K	P	L	K	L	Q	V	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	G	T	GI 163952		
317	N	A	S	Q	L	S	R	T	Q	-	-	S	R	E	P	R	Q	-	Q	E	E	K	P	L	K	L	Q	V	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	G	T	GI 1355759	
320	N	A	S	Q	L	S	R	T	Q	-	-	S	R	E	P	R	Q	-	Q	E	E	K	P	L	K	L	Q	V	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	G	T	GI 184088	
320	N	N	S	Q	L	N	R	S	Q	-	-	C	Q	E	P	R	W	-	Q	E	D	K	P	L	N	L	Q	V	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	G	T	GI 791239
319	N	N	S	L	L	P	R	S	Q	-	-	S	R	E	G	R	W	-	Q	E	E	K	P	L	K	L	Q	V	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	G	T	GI 236184
370	Q	H	K	D	M	V	R	I	P	V	G	S	R	E	T	F	Y	-	R	I	S	K	T	D	G	V	C	E	W	K	F	F	S	S	M	P	R	G	S	A	GI 927211								
428	F	K	K	H	G	I	R	N	-	-	G	I	N	P	A	M	Y	-	Q	S	P	M	R	L	R	S	S	T	I	Q	S	S	S	I	I	L	L	D	T	L	GI 475198								

FIGURE 2D

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1722180
GI 163952
GI 1355759
GI 184088
GI 791239
GI 236184
GI 927211
GI 475198

421	H	C	T	C	P	P	K	R	R	S	S	V	T	F	E	D	E	V	E	Q	I	K	E	A	A	K	N	F	D	S	S	C	E	S
349	E	V	T	A	P	R	G	A	T	D																								
346	E	V	T																															
349	E	V	T	A	P	Q	G	A	T	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R		
349	E	V	T	A	P	Q	G	A	T	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R		
348	E	L	T	H	P	Q	G	N	P	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R		
409	R	I	T	V	S	K	D	Q	S	S	C	T	T	A	R	G	H	T	P	M	T													
465	L	L	T	E	N	E	G	D	K	T	-	-	-	-	E	Q	V	S	Y	V														

FIGURE 2E

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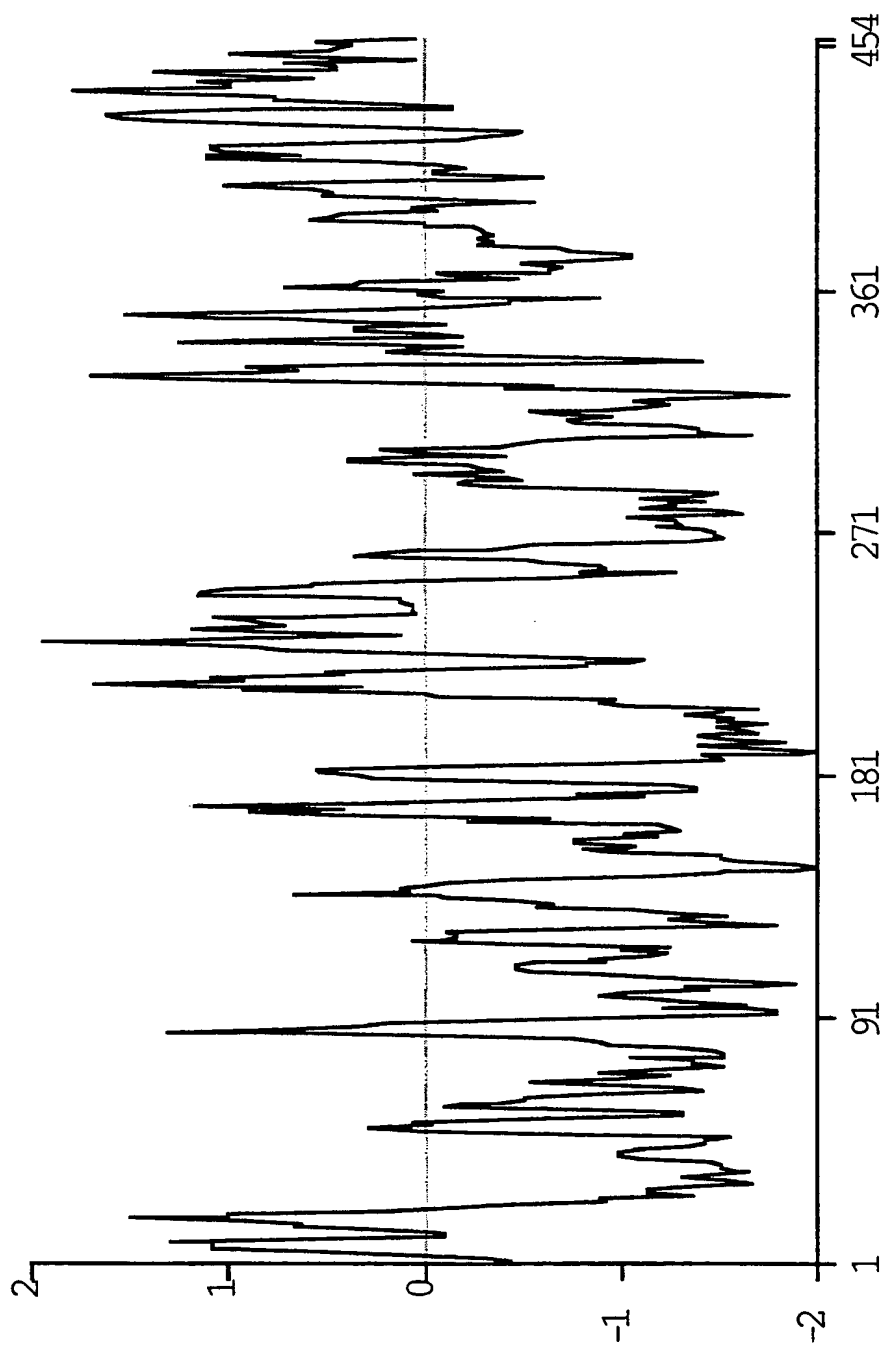


FIGURE 3

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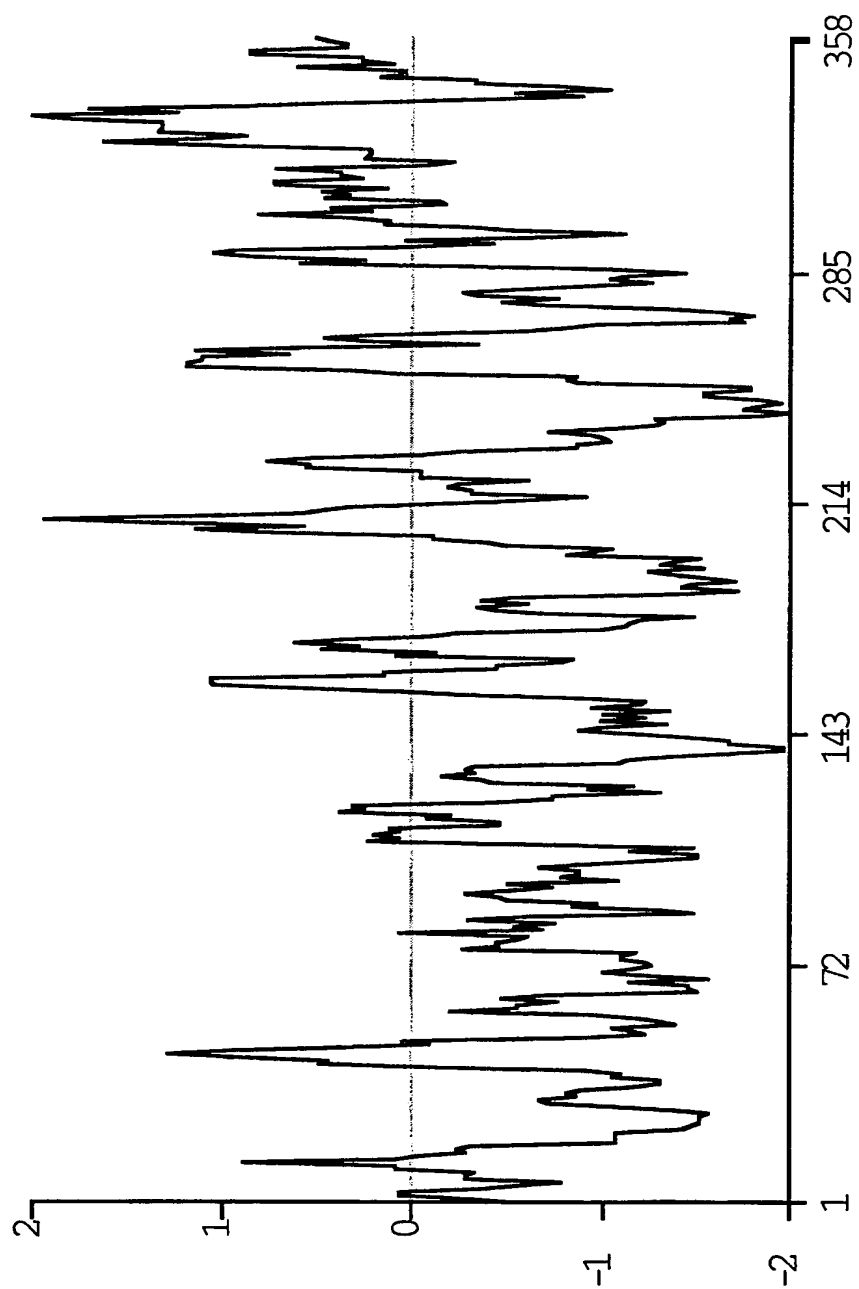


FIGURE 4

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/20200

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K14/705 C12N15/09 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

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IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 05225 A (HUMAN GENOME SCIENCES INC ;SOPPET DANIEL R (US); LI YI (US); ADAMS) 22 February 1996 see claims 1,4,21,22; figure 1	1-11,15, 19
Y	see claims 1,4 ---	1-20
Y	WO 94 05695 A (UNIV NEW YORK) 17 March 1994 see page P2, line 14 - line 21 -----	1-20

☐ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

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Date of mailing of the international search report

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WO 9605225 A	22-02-96	AU 7669394 A EP 0773959 A	07-03-96 21-05-97
WO 9405695 A	17-03-94	AU 4855393 A US 5508384 A	29-03-94 16-04-96

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶: C07K 14/705, C12N 15/09, A61K 38/17	A1	(11) International Publication Number: WO 98/20040 (43) International Publication Date: 14 May 1998 (14.05.98)
(21) International Application Number: PCT/US97/20200 (22) International Filing Date: 5 November 1997 (05.11.97) (30) Priority Data: 08/748,485 7 November 1996 (07.11.96) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 08/748,485 (CIP) Filed on 7 November 1996 (07.11.96) (71) Applicant (for all designated States except US): INCYTE PHARMACEUTICALS, INC. [US/US]; 3174 Porter Drive, Palo Alto, CA 94304 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): AU-YOUNG, Janice [US/US]; 1419 Kains Avenue, Berkeley, CA 94702 (US). GUEGLER, Karl, J. [CH/US]; 1048 Oakland Avenue, Menlo Park, CA 94025 (US). GOLI, Surya, K. [IN/US]; 620 Iris Avenue #338, Sunnyvale, CA 94086 (US). MURRY, Lynn, E. [US/US]; 1124 Los Trancos Road, Portola Valley, CA 94028 (US).		(74) Agent: BILLINGS, Lucy, J.; Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304 (US). (81) Designated States: AT, AU, BR, CA, CH, CN, DE, DK, ES, FI, GB, IL, JP, KR, MX, NO, NZ, RU, SE, SG, US, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: NOVEL HISTAMINE H2 RECEPTOR (57) Abstract The present invention provides a novel histamine H2 receptor (H2RH) and polynucleotides which identify and encode H2RH. The invention also provides genetically engineered expression vectors and host cells comprising the nucleic acid sequences encoding H2RH and a method for producing H2RH. The invention also provides for agonists, antibodies, or antagonists specifically binding H2RH, and their use, in the prevention and treatment of diseases in which H2RH is expressed. Additionally, the invention provides for the use of antisense molecules to polynucleotides encoding H2RH for the treatment of diseases associated with the expression of H2RH. The invention also provides diagnostic assays which utilize the polynucleotide, or fragments or the complement thereof, and antibodies specifically binding H2RH.		

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